UNIVERSITY OF EDINBURGH

PHYSICO-CHEMICAL STUDIES ON MATERIALS CONTAINING URONIC ACIDS; WITH SPECIAL REFERENCE TO DECARBOXYLATION

- by -

SIDNEY GARBUTT

THESIS

submitted for the degree of

DOCTOR of PHILOSOPHY

August, 1960

CONTENTS

FIGURE INDEX

ACKNOWLEDGMENTS

I am indebted to the Department of Scientific and Industrial Research for the provision of a Research Scholarship.

Thanks are also due to Professor E.L. Hirst, C.B.E., F.R.S. for his interest in the work and for the provision of laboratory facilities.

I would also like to take this opportunity to thank the staff and fellow students of this department whose friendship has contributed so much to the enjoyment of my studies in Edinburgh.

I would particularly like to thank my supervisor, Dr. D.M.W. Anderson, whose advice, knowledge and help have been a continuous source of encouragement to me throughout this work.

Department of Chemistry,
The University,
Edinburgh, 9.

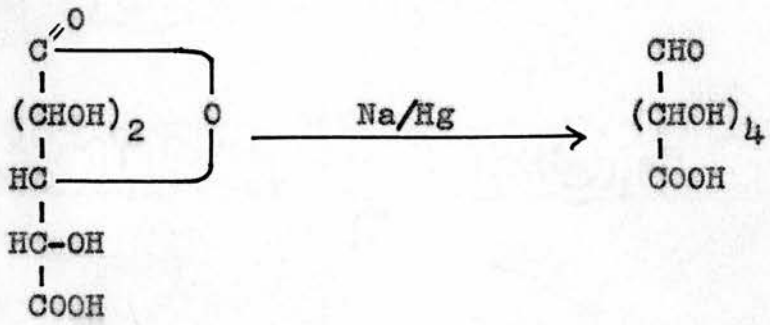
August 1960

GENERAL INTRODUCTION

A uronic acid is produced when the primary alcohol group in a simple sugar is replaced by a carboxy acid group. Although uronic acids derived from tetroses are known (1), they have attracted little attention to date and most investigators have studied the acids obtained from pentoses and hexoses.

The most common synthetic methods of obtaining uronic acids (II) involve (2)(3) either the reduction of the monolactone of a saccharic acid (I)

e.g.



I II

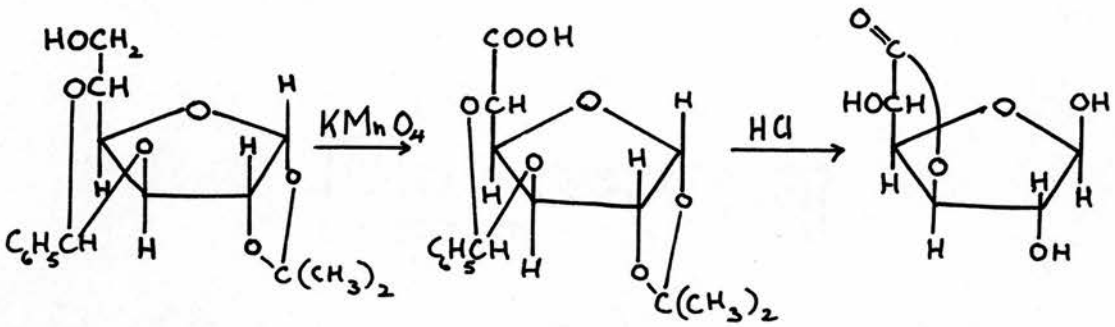
or the oxidation of the primary alcohol group

e.g.

D-galactose D-galacturonic acid

III IV

In most cases the oxidation cannot be achieved directly from the unsubstituted sugar; condensation products of the sugar with aldehydes and ketones are therefore the usual starting materials. e.g. Glucurone (VII) the lactone of glucuronic acid, is obtained from 1-2-monoacetone 3-5-benzylidene glucose (V) via VI.



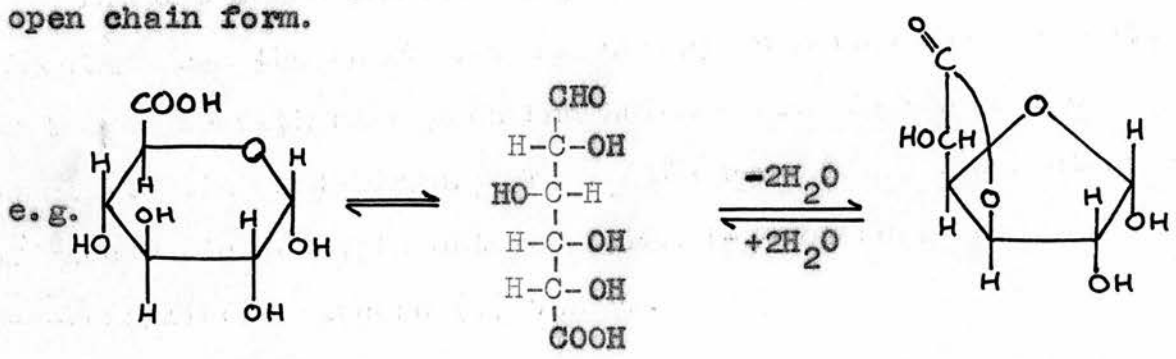
1-2-Monoacetone 3-5-benzylidene glucose

Glucurone (β-form)

V VI VII

With certain of the uronic acids, e.g. glucuronic and mannuronic acids, the configuration of the molecule is such that an internal condensation, with resultant loss of one molecule of water readily occurs, and the corresponding lactone is produced. This presumably takes place via the

open chain form.



α-pyranose form — VIII

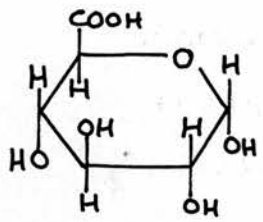
Open chain form — IX

Glucurone — X

With α-D-galacturonic acid no lactonisation is possible because of the instability of a combined 5 and 6 membered bicyclic system.

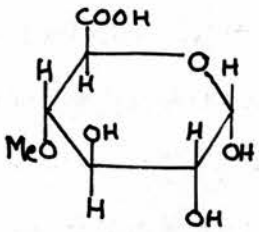
In solution, an equilibrium exists between the acid and the lactone, and this can cause analytical difficulty: in chromatographic separations, for instance, the acid and the lactone behave in quite different ways. In other operations, however, the fact that some uronic acids form lactones whilst others do not can be used to advantage.

Until recently, only glucuronic (XI), 4-O-methyl-glucuronic (XII), galacturonic (XIII) and mannuronic (XIV) acids had been found to occur naturally in polysaccharide materials (4) and these are the acids which have been most extensively studied. Of these, mannuronic acid is very difficult to prepare in reasonable quantity in pure form.



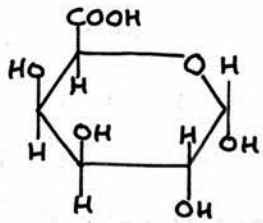
α-D-Glucuronic-

XI



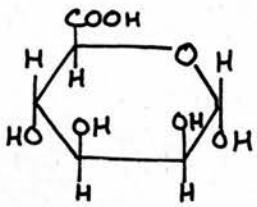
4-O-methyl-glucuronic-

XII



Galacturonic-

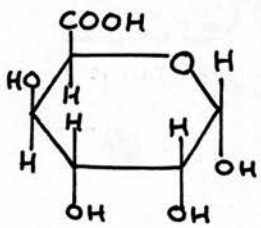
XIII



Mannuronic-

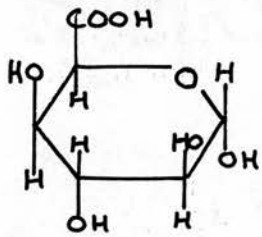
XIV

In the last two or three years, however, guluronic (XV) (35) and iduronic (XVI) (5) acids have been isolated from natural products in which their presence had not earlier been detected. The presence of D-lyxuronic acid (XVII) as a glucose metabolite in micro-organisms has also been reported (6).



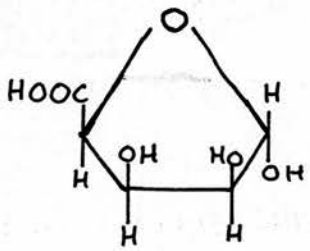
α-Guluronic-

XV



α-Iduronic-

XVI

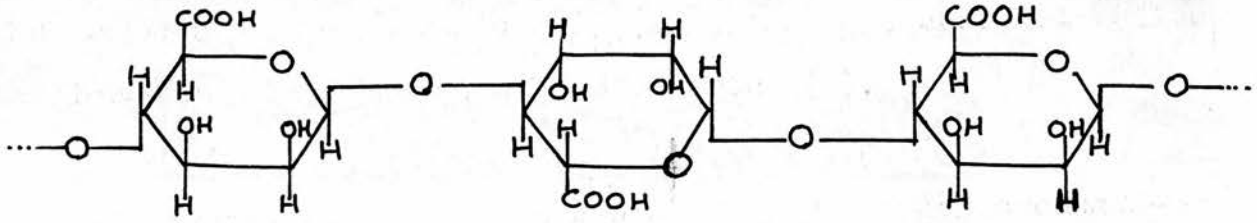


α-Lyxuronic-

XVII

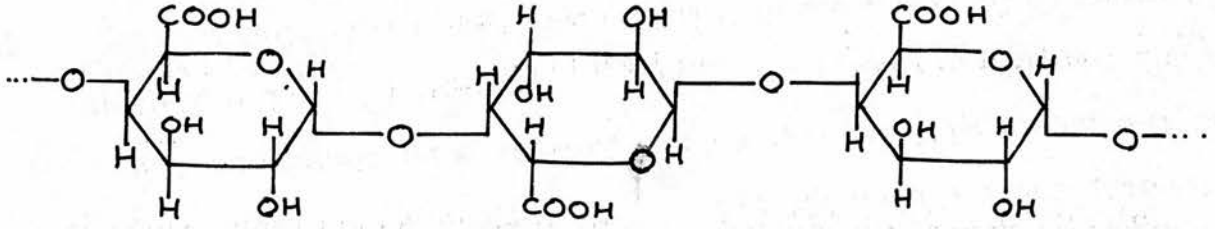
Previously regarded as chemical curiosities, these uronic acids are now of very great interest and importance, although at the present time authentic samples of these compounds are particularly difficult to obtain for reference purposes.

The number of uronic acids occurring in nature are therefore few, and they seldom, if ever, occur uncombined. Glucuronic-, galacturonic- and mannuronic acids are, however, commonly found as residues or units in polysaccharide systems of both plant and animal origin. Approximately one-third of the polysaccharide systems which have been characterised contain uronic acid residues, often, but not always, as end groups. The percentage of uronic acid groups in such polymer systems varies widely, from a few percent in certain plant gums and also in polymers of the hemicellulose group, to 100% in the case of pure alginic acid (XVIII), which is therefore correctly termed a polyuronide.



Alginic acid: - β-D-mannuronic acid units, linked -1:4-

(XVIII)

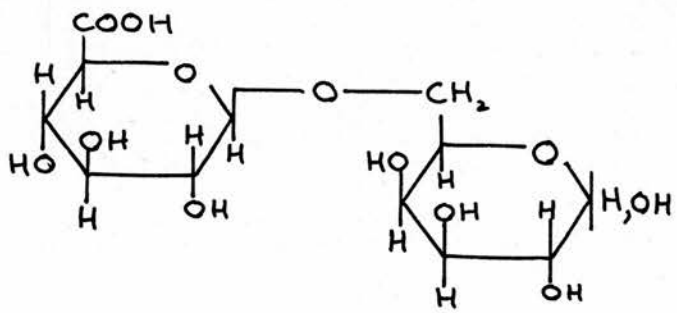


Pectic acid: - α-D-galacturonic acid units, linked -1:4-

(XIX)

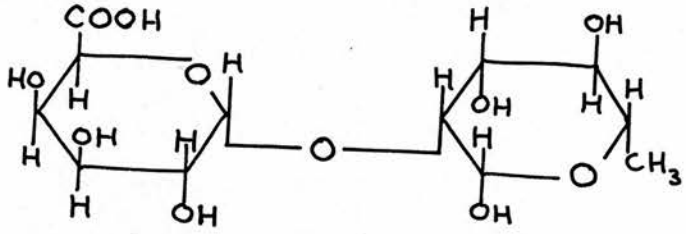
In polyuronides, the chemical linkages between adjacent uronic acid residues are so stable and resistant to hydrolysis that the drastic conditions necessary to cleave the glycosidic linkages cause simultaneous destruction of the monomer units as they are produced. It is for this reason that the preparation of pure mannuronic acid from alginic acid is very difficult.

Likewise, in polysaccharides, chemical bonds between uronic acid residues and the adjacent pentose or hexose are also resistant to hydrolysis. This property is often used in the structural analysis of polysaccharides. Graded hydrolysis using weak mineral acid (e.g. 1% H_2SO_4 at 100°C) (7) often yields aldobiuronic acids. These are disaccharides, in which a uronic acid is attached to a pentose or hexose by the same glycosidic linkage as was present in the polymer. Thus the aldobiuronic acid 6-O-(β-D-glucopyranosyluronic acid)-D-galactose (XX) has been isolated from gum arabic (8),



XX

and 2-O-(D-galactopyranosyluronic acid)-L-rhamnose (XXI) was obtained from Slippery Elm mucilage.

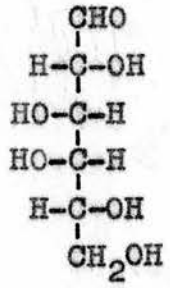


XXI

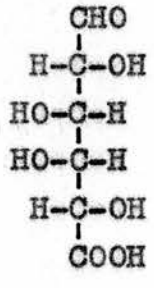
The isolation of aldobiuronic acids is of the greatest importance in helping to elucidate the structure of the polysaccharide. Recently (9) catalytic oxidation of structurally interesting polysaccharides has been used, particularly in the elucidation of pentosan structure, to give aldobiuronic acids.

It is now recognised that two groups of sugars are commonly found in co-existence in plant polysaccharides.

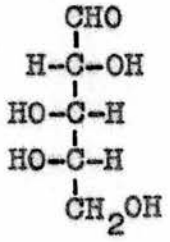
These are (a) D(+)-galactose (XXII), D(+)galacturonic acid (XXIII), L(+) arabinose (XXIV),



XXII

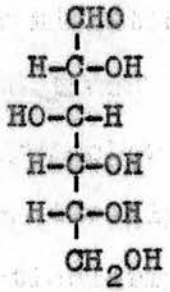


XXIII

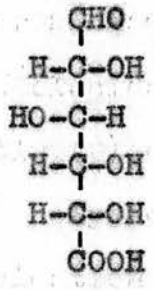


XXIV

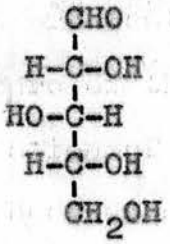
and (b) D(+) glucose (XXV), D(+) glucuronic acid (XXVI), D(+)xylose (XXVII).



XXV



XXVI



XXVII

In each group it will be seen that the sugars have the same internal configuration. This has led to the suggestion that the pentoses in nature may be derived from the hexoses by oxidation at C-6 to hexuronic acid, followed by decarboxylation. In polysaccharides, this is clearly not a polymer to polymer transformation, since pure xylans with no uronic acid have been

isolated (10). Recent work (11) with labelled sugars has shown that D-glucurono-lactone-1-C^{14} is equally effective as D-glucose as a xylan precursor; D-xylose having about 90% of the isotopic carbon at C-1 can be isolated. The actual intermediates in polysaccharide formation, while thought to be phosphate derivatives (12), have not been characterised.

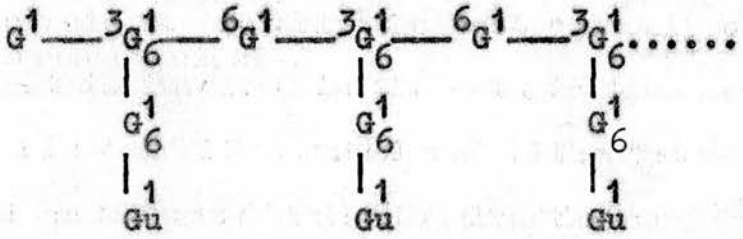
Most polysaccharides are easily degraded by both acid and alkali, and preparative methods are therefore usually based on complexing reactions. Ca^{++} salts have been used to precipitate pectins, alginates, and mucopolysaccharides; Al^{+++} salts are used in the commercial preparation of pectins. More recently, complexing with quaternary ammonium salts has yielded undegraded products (13). The salts are formed in neutral solution as precipitates which decompose in strong salt solutions (e.g. 10% $MgCl_2$ or NaCl), to give the undegraded polysaccharide.

Glucuronic acid is probably the most commonly occurring uronic acid. It is involved in the detoxication mechanism of mammals. Animals fed with borneol and with glucose labelled with C^{14} at C-1 or C-6 were found to give the conjugate bornyl glucosiduronic acid with most of the activity at carbons 1 and 6 respectively (14). Pure D-glucuronic acid can be prepared from the conjugate glucuronide by hydrolysis, followed by precipitation of the barium salt, from which the free glucuronic

acid is obtained by treatment with dilute acid (15). More recently, cation exchange resins have been used to remove the metal ions from solution to give the pure acid (16).

While glucuronic acid has been found in plant structural polysaccharides, e.g. cell-wall xylan from pear wood (17) and from wheat straw (18), it does not appear to occur in the reserve polysaccharides of plants.

Glucuronic acid occurs commonly in plant gums, which are the natural exudates produced to resist bacterial and parasitic invasion when an injury has been sustained by the tree or plant. The gum produced by grape-fruit, lemon, cherry, damson and plum trees have all been shown to contain glucuronic acid, which is also present in the gum of greatest commercial importance, Gum Arabic (8). The structure of the repeating unit of degraded arabic acid, obtained from gum arabic, is shown below.



G = D-galactopyranose Gu = D-Glucopyranosyluronic acid

This gum is formed when trees of the Acacia species, particularly A.senegal, are deliberately tapped by tearing short strips off the bark. Gum arabic is of great economic importance in countries such as the Sudan, which produces 40 million pounds per annum.

4-O-Methyl-D-glucuronic acid occurs in Mesquite gum (19) and Gum Myrrh (20), and is also a common constituent of the hemicelluloses from various trees (21).

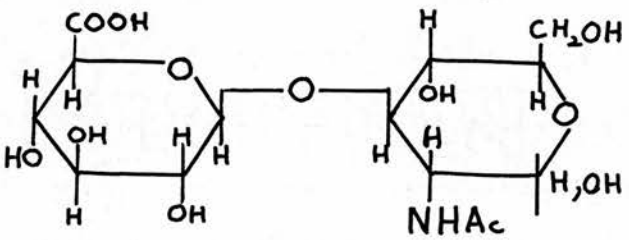
Glucuronic acid was reported to occur in alginic acid, a polymannuronic acid obtained in good yield from certain seaweeds. This report has not been confirmed, but glucuronic acid has been found in the cell-wall of the fresh-water algae, Nitella translucens and Chara australis (22).

Glucuronic acid is also present in many polysaccharides of animal origin. It is found, together with the sulphate ester of D-glucosamine, in the blood anticoagulant heparin (23).

Chondroitin sulphates Types A and C, which, along with the protein collagen, are some of the major constituents of cartilage, yield equimolar amounts of D-glucuronic acid, 2-deoxy-2-amino-D-galactose sulphuric acid and acetic acid on hydrolysis (24). L-Iduronic acid is thought to be the uronic acid present in Chondroitin sulphate Type B in which the sulphate ester linkages are at C-4 of the galactosamine residues, with the L-iduronic acid residue linked at C-3 (25).

The important mucopolysaccharide, hyaluronic acid, which was

first isolated from vitreous humor, occurs in 1-2% amounts in human umbilical cord, and can be isolated as a protein-polysaccharide complex. The polysaccharide appears to contain equimolar proportions of D-glucuronic acid and N-acetyl-D-glucosamine (26); the hyalbiuronic acid isolated has been identified as 3-O-(β-D-glucopyranosyluronic acid)-2-amino-2-deoxy-D-glucose (XXVIII) (27).



XXVIII

The presence of an amino-uronic acid in animal polysaccharides and glycoprotein, although readily conceivable, has not been established. Types II, III and VIII pneumococcus-specific polysaccharides also contain glucuronic acid, but Type I contains galacturonic acid.

Galacturonic acid is found in some plant gums, e.g. Gum karaya (28) and Gum tragacanth (29) which are produced in 10 million lb. quantities and are therefore of commercial importance. Even more important, however, are the pectins and pectic acids which are widely used in foodstuffs (30).
[A pectic acid (XIX) chain is shown on p.5.]

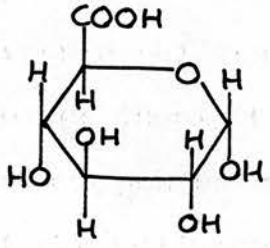
In pectins it is usual to find that a proportion of the galacturonic acid units in the polymer chain exist in the methyl ester form. Pectin contains up to 80% galacturonic acid depending on the source. The free galacturonic acid may be obtained by hydrolysis or by digestion with a suitable pectic enzyme (31). Di- and tri-galacturonic acids have also been prepared by incomplete enzymic degradation of pectin followed by separation on ion-exchange resin (32).

Galacturonic acid forms sodium-calcium and sodium-strontium-D-galacturonates, which facilitate the separation of α-D-galacturonic acid from the hydrolytic liquor. These double salts, which appear to be unique in the carbohydrate field, contain one atom of a monovalent and one atom of a divalent metal in combination with three α-pyranose galacturonate residues. They are generally less soluble than normal salts and crystallise exceptionally well. The salts give pure galacturonic acid on hydrolysis with oxalic acid and crystallisation (33).

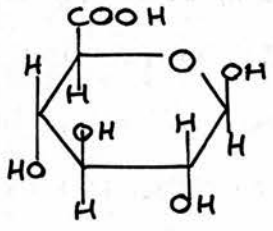
Mannuronic acid occurs in hemicelluloses, but the main natural source is alginic acid which can be extracted in good yield from certain types of seaweed. Alginic acid was originally believed to be a linear β-1:4 polymer of mannuronic acid units. [Alginic acid (XVIII) is shown on p.5.] This polymer is extremely resistant to acid hydrolysis, and quantitative yields of mannuronic acid could not be obtained (34). In recent work (35), guluronic acid has been isolated from alginic acid in 12%

yield; failure to detect this in earlier studies has been explained by the rapid epimerisation (on C-5) of the L-guluronic acid to D-mannuronic acid. In even more recent work (36) the use of 96% H_2SO_4 at 5°C., followed by 0.5N.H_2SO_4 for 5 hrs. at 95°C., gave mannurone (15%), mannuronic acid (37.2%), guluronic acid (10%), and gulurone (2%), although few workers appear to be able to repeat these high yields owing to the large amount of decomposition and decarboxylation of the uronic acid residues. Very recently the fractionation of alginic acid has been carried out using potassium chloride and manganese chloride solutions (37); two main fractions, differing in the ratios of guluronic acid to mannuronic acid, were obtained.

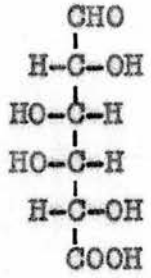
Theoretically, uronic acids can exist in the pyranose, furanose, or open chain forms; as α- and β-modifications; and stereochemically in the boat or chair conformations. These are shown in the case of glucuronic acid.



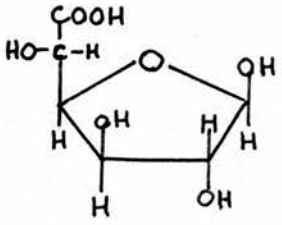
α-D-glucopyranosyl uronic acid



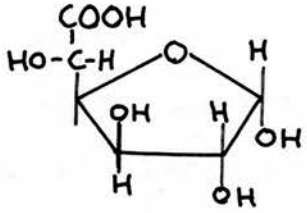
β-D-glucopyranosyl uronic acid



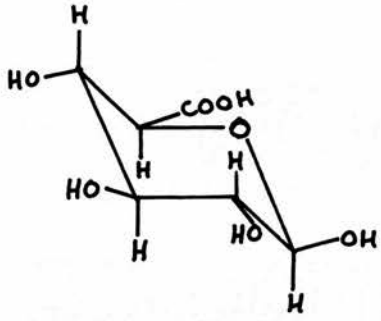
D-Glucuronic acid
(open-chain form)



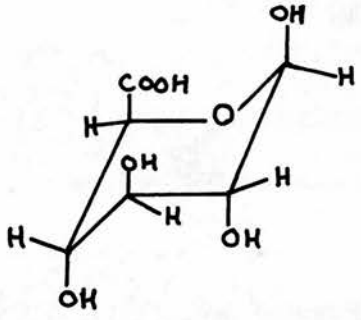
β-D-glucofuranosyl
uronic acid



α-D-glucofuranosyl
uronic acid



β-D-glucuronic acid
(Chair form - C1 conformation)



β-D-glucuronic acid
(Chair form - 1C conformation)

Two crystalline modifications of galacturonic acid, both having the pyranose structure, were shown to exist (38). These gave mutarotations resembling those of α- and β-D-galactose. The salts, both simple and double, also showed this mutarotation (39), the α-form starting with a high +ve rotation which decreases. Galacturonic acid, obtained by hydrolysis of a pectin, gives the α-form, which recrystallises from aqueous solution or dilute ethanol to give the monohydrate. The β-form is obtained by boiling the monohydrate in absolute alcohol and rapidly concentrating the solution.

Both α- and β-mannuronic acids have been isolated (40) by the decomposition of Ba-D-mannuronate (i) at -10°C. for the β-acid and (ii) at room temperature for the α-form. Glucuronic acid appears to have been prepared in the β-form only (41) by crystallisation from 90% alcohol. The crystalline γ-lactones of glucuronic acid and mannuronic acid have been prepared and are known as glucurone (42) and mannurone (43), both being more stable than the parent acids. Although they are thought to be in one particular form in the solid state, it has not been reported whether it is α- or β-.

Recently, (44), the epimerisation, at C-5, of D-uronic acids to the L-form, by heating in aqueous solution at pH 7 for 2 hrs. at 100°C. has been studied; up to 50% conversion to the epimer was found. This, then, provides a convenient method for the preparation of L-series uronic acids, and would also explain, to some extent, the present confusion with regard to the structure of alginic acid.

The separation of mixtures and qualitative identification of uronic acids can be most conveniently carried out by paper partition chromatography. Since the lactones contain fewer -OH groups, these run more quickly than the free acids. Various solvents have been used, most of them acidic. Pyridine:ethyl acetate:acetic acid:water (5:5:1:3) is recommended for acids, and pyridine:ethyl acetate:water (11:40:6) for lactones (35).

Various other solvents are also recommended, e.g. (a) butan-1-ol:acetic acid:water (4:1:5), (b) butan-1-ol: formic acid:water (500:115:385) (45), and (c) ethyl acetate: acetic acid:formic acid:water (18:3:1:4) (46).

The spots are detected by spraying the chromatogram with aniline oxalate/acetic acid or with any other reagent, e.g. p-anisidine-HCl (47), which reacts with the aldehydic or carboxyl group to give a coloured compound; heating at approx. 120°C. is generally required for colour development. Larger quantities can be separated on chromatographic columns or on thick chromatography paper; the columns are usually of cellulose for monomers, charcoal for oligosaccharides, and celite-magnesol for acetylated sugars (46). Uronic acids can also be separated by electrophoresis (48). Characterisation of uronic acids can be carried out by forming salts with alkaloids e.g. cinchonine and brucine: the hydrazones are difficult to obtain free from mixtures of hydrazide and osazone, although the 2:4-dinitrophenylhydrazone appears to be satisfactory (49). Oxidation to the dibasic acid has been used particularly in the case of galacturonic acid, which gives mucic acid.

At present the quantitative determination of uronic acids is based on methods involving either colorimetry or decarboxylation. None of the available methods is specific for a particular uronic acid, and many side reactions and complicating

factors occur in each of the known procedures. At the outset of this work, many conflicting reports concerning each of the available methods of analysis were to be found in the literature.

This brief introduction, whilst being far from exhaustive, will have given an indication of the widespread occurrence of uronic acid residues in material of both plant and animal origin, which are of very great commercial and biological importance. It was therefore considered of interest to undertake a study of the physico-chemical and analytical behaviour of uronic acids in an attempt to make the methods for their quantitative determination more reliable and accurate.

PART I

The kinetics of the decarboxylation of Uronic Acids and closely related compounds

INTRODUCTION.

(i) General decarboxylation mechanisms.

Decarboxylation is the replacement of the carboxyl group of an acid by hydrogen. The reaction is widely used in preparative organic chemistry and a large number of suitable reagents are available (50). The strength of the R-COOH bond, where R is any organic group, varies within wide limits. While the fatty acids totally decompose before decarboxylation, a large number of other carboxylic compounds are known which can be decarboxylated either at room or elevated temperatures or under acid or alkaline conditions: one mole of CO_2 is produced and the rest of the molecule is left intact (51)

$$\text{i.e.} \qquad R\text{-}COOH = RH + CO_2.$$

If the electron density of the α-carbon atom is low, then it will have high electron affinity. The reaction will take place easily and will follow a unimolecular course. This will therefore be a unimolecular electrophilic substitution (S_E1 type reaction) (52).

$$\text{(a)} \quad R\text{-}COOH \rightleftharpoons RCOO^- + H^+$$

$$\text{(b)} \quad RCOO^- = R^- + CO_2$$

$$\text{(c)} \quad R^- + H^+ = RH.$$

The rate of reaction (b) will be rate determining, the reaction being favoured when R is a good proton acceptor, as in trichloroacetic acid and nitroacetic acid, and when a solvent of high dielectric constant is used. There is, as yet, no evidence regarding the form of the $-\overset{|}{\underset{|}{C}}-COOH$ group on decarboxylation although three possibilities may be considered

i.e. (a) $R-C(=O)-OH \longrightarrow CO_2 + RH$

(b) $R^- + CO_2$

(c) $HR + CO_2$

A modification of (a) is possible when the solvent (S) also takes part in the reaction.

i.e. $-\overset{|}{\underset{|}{C}}-C(=O)-O-H + S \longrightarrow -\overset{|}{\underset{|}{C}}^- + CO_2 + HS^+$

Some compounds, e.g. trihalogenoacetic acids, may display types (b) and (c) simultaneously.

The main evidence for a unimolecular mechanism for these compounds is:

(1) They are almost completely ionised in water.

(2) The activation energies for the decarboxylation of these acids and their completely ionized salts are identical.

(3) Decarboxylation is very much slower in non-polar solvents such as toluene than in water.

(4) In non-aqueous solvents, in which the acids are only slightly ionized, the rate is proportional to the concentration of the anion produced by added bases.

e.g. $Cl_3C.COOH + B \rightleftharpoons Cl_3C.COO^- + BH^+$.

In contrast a bimolecular reaction is favoured (53) if the electron density of the α-carbon atom is high. Reactions of this kind are catalysed by both acids and bases, and proceed either pseudounimolecularly or bimolecularly (S_E2 type reaction).

(a) (i) $H^+ + RCOOH \rightleftharpoons H^+ - - - R - - - COOH$

(ii) $H^+ - - - R - - - COOH = RH + CO_2 + H^+$

or (b) $H^+ + RCOO^- = HR + CO_2$.

In general, a change in rate following a change in hydrogen ion concentration therefore indicates a bimolecular reaction (but see p. 76).

(ii) General decarboxylation methods.

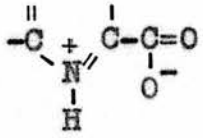
The simplest way to effect decarboxylation is to heat the carboxylic acid alone or in aqueous solution. Trichloroacetic acid, for example, decomposes easily in aqueous solution at

70°C. to give chloroform and CO_2.

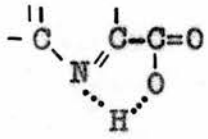
i.e. $CCl_3COOH = CHCl_3 + CO_2$.

This reaction is accelerated by the addition of organic bases, e.g. aniline, to a benzene solution of trichloroacetic acid; similarly, by heating in dimethyl aniline, pyridine, or quinoline there is smooth formation of chloroform and carbon dioxide. This reaction is quite general; many carboxylic acids decarboxylate under these conditions. Nitrogen and oxygen heterocyclic α-carboxylic acids, however, decarboxylate easily, by a first order mechanism, in glacial acetic acid + 10% acetic anhydride or by heating with copper powder. This is because the high electron affinity of the heterocyclic atom gives a low electron density at the α-carbon atom.

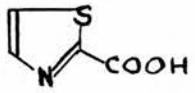
Two structures for the decarboxylating acid are possible, the zwitterion, XXIX, and the chelate type, XXX.



XXIX



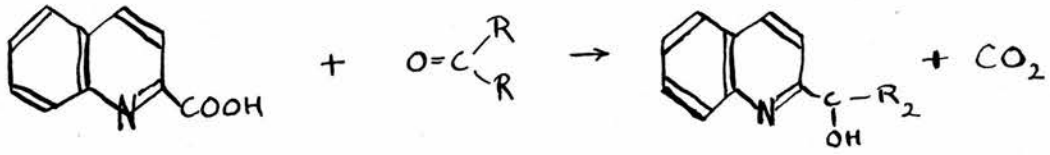
XXX



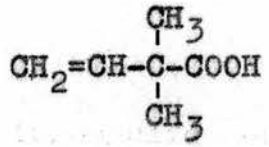
XXXI

The respective concentrations of each determine the rate of decarboxylation. Thiazole-2-carboxylic acid, XXXI, which decarboxylates in quinoline at room temperature, has two hetero-atoms, both with high electron density, α- to the carboxyl group.

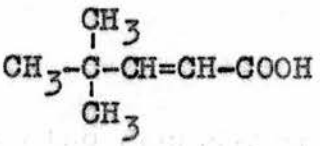
Heterocyclic α-carboxylic acids also decarboxylate when boiled with aldehydes and ketones; a reaction analogous to an aldol condensation takes place.

i.e. 

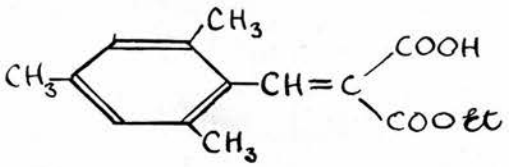
α-β-Unsaturated acids decarboxylate by a first order mechanism, the reaction being easily carried out in the presence of copper oxide as catalyst at 140°C. It is suggested that β-γ-unsaturated acids are formed as intermediates in these reactions, since α-β-unsaturated acids in which isomerisation to the β-γ-form is impossible show marked resistance to decarboxylation. e.g. 2,2-dimethyl buten-3-oic acid, XXXII, decarboxylates to give 2-methyl butene-2, whilst 4,4-dimethyl pentene-2-oic acid, XXXIII, does not easily decarboxylate.



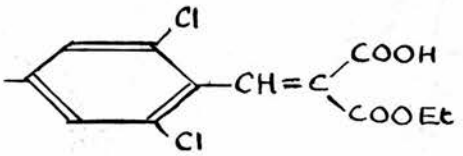
XXXII



XXXIII



XXXIV

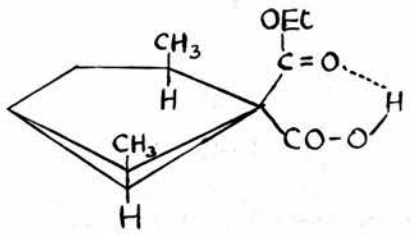


XXXV

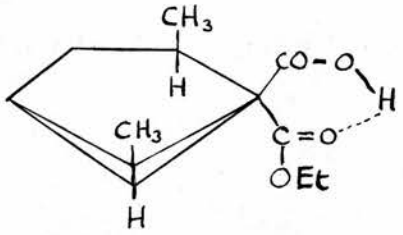
The direct decarboxylation of α-β-unsaturated malonic acid derivatives is much slower when no isomerisation to the β-γ-derivative is possible: ethyl hydrogen mesitylidene malonate, XXXIV, and ethyl hydrogen 2-6-dichlorobenzylidene malonate, XXXV, neither of which can isomerise to the β-γ-intermediate, do not readily lose carbon dioxide (54).

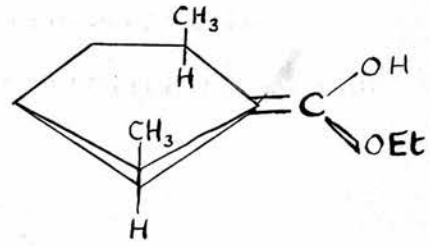
There is evidence that the decarboxylation of the β-γ-unsaturated acids proceeds via a chelated intermediate (55). The fact that the isomeric half esters of 2,5-dimethylcyclopentane dicarboxylic acid, XXXVI, XXXVII, decarboxylate to yield the same mixture of monoesters indicates that a common intermediate is involved in both processes.

XXXVI



XXXVII





XXXVIII

α-Hydroxy carboxylic acids, e.g. citric acid, decarboxylate and dehydrate on heating in concentrated sulphuric acid, giving the appropriate aldehyde.

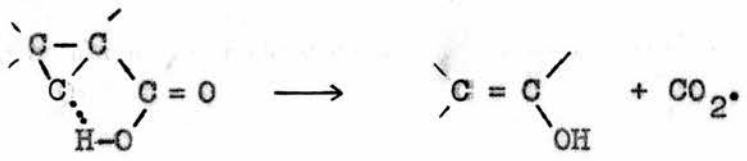
i.e. $RCH(OH)COOH = RCHO + CO_2 + H_2O$.

α-Keto acids and glycidic acids are also decarboxylated in hot 10% NaOH to produce aldehydes.

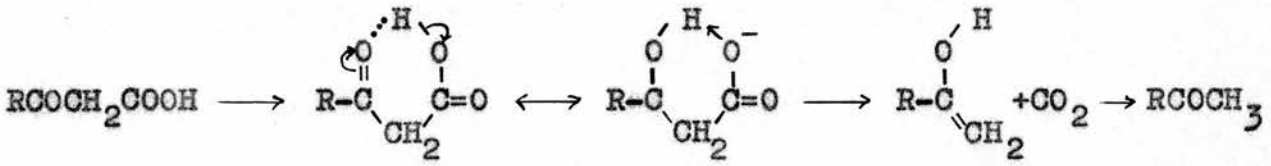
e.g. $R\text{-}CO\text{-}COOH = R\text{-}CHO + CO_2$.

$$R\text{-}\underset{\diagdown O \diagup}{CH\text{-}CH}\text{-}COOH = RCH_2CHO.$$

This again can be represented as taking place via a chelate ring.

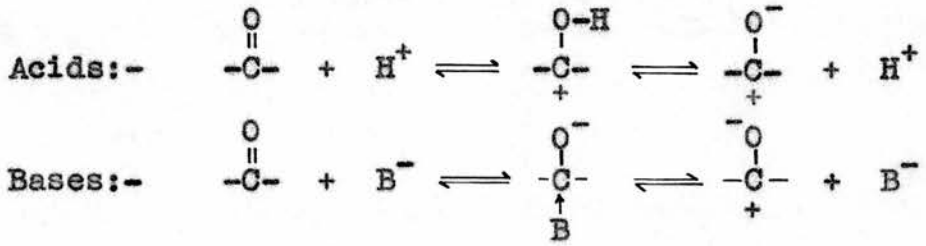


The decarboxylation of a β-keto carboxylic acid proceeds by a second order reaction to give a ketone and carbon dioxide (53); cyclic chelated intermediates are again postulated.

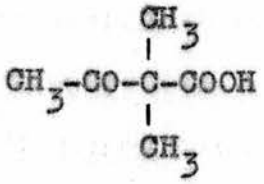


XXXIX XL XLI XLII XLIII

The cationic centre in the β-position, i.e. the carbonyl group, is chelated to the acidic hydrogen of the carboxyl group to give the active transition state, XLI. This decomposes by a simple electromeric shift to give the enol which by tautomeric shift gives the ketone (56) (57). The reaction is catalysed by acids and also weak bases, the carbonyl group being activated.

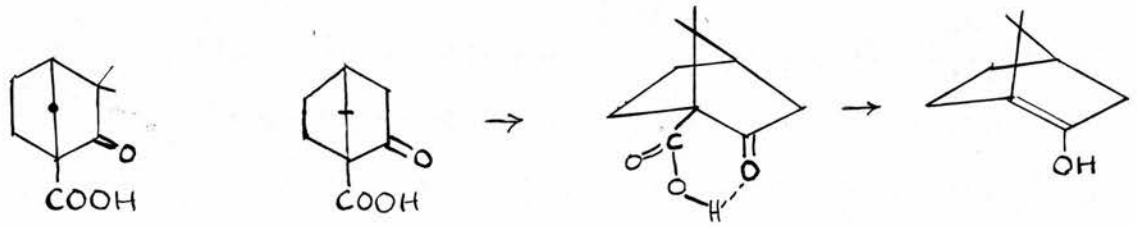


In aqueous solution the reaction can be self catalysed, but in acid an S_E2 bimolecular reaction occurs. Studies on the decarboxylation of β-keto acids have shown that it is the keto form of the parent acid which undergoes loss of carbon dioxide, so that the reaction is independent of the capacity to enolise.



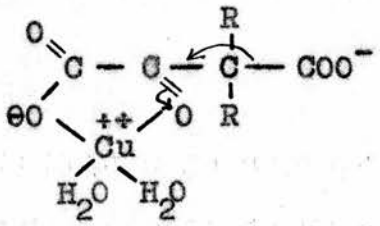
XLIV

α-α'-dimethyl acetoacetic acid, XLIV, which cannot form an α,β- double bond, decarboxylates four times as fast as the unsubstituted aceto acetic acid. The rate of the uncatalysed decomposition is independent of the dielectric constant of the solvent, showing that the rate determining step does not involve a highly polar intermediate. It is therefore postulated that the decarboxylation takes place through the hydrogen bonded form of the parent β-keto acid. Where the formation of a double bond and enol involves considerable strain, the parent acid is found to be resistant to decarboxylation. Both camphenonic acid, XLV, and ketopinic acid, XLVI, are resistant to decarboxylation under vigorous thermal conditions due to the fact that the formation of a ring system involves a large amount of strain.



XLV XLVI XLVII XLVIII

Cyclic complexes, XLIX, have been postulated for the decarboxylation of oxaloacetic acids by metal ions (58)



XLIX

Dextro-camphocarboxylic acid has been reported (59) to decarboxylate more rapidly in the presence of optically active L-bases than with D-bases present.

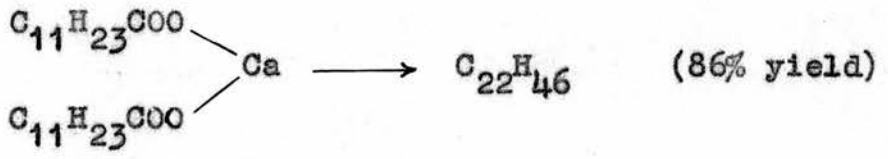
The loss of carbon dioxide from silver salts of carboxylic acids on treatment with bromine seems to be independent of steric factors; acids stable to thermal decarboxylation are found to decarboxylate readily when the silver salt is brominated. Price (60) has suggested a radical mechanism, and in fact, most of the reactions investigated appear to be non-ionic.

In general, simple aromatic carboxylic acids in which the carboxyl group is not ortho- or para- to a hydroxyl group or heterocyclic atom are not decarboxylated by the methods described. However, pyrolysis of their salts, such as the calcium salt, with excess soda-lime, leads to the complete elimination of the carboxyl group: the barium salts, and occasionally silver and mercury salts have also been used. The principal application of this method is to the decarboxylation of aromatic acids, some

fatty acids, and some alicyclic carboxylic acids, although the latter tend to dehydrogenate.

The calcium or barium salts of the fatty acids, on dry distillation, undergo partial decarboxylation to ketones.

Electrolysis of the sodium salt gives the hydrocarbon (Kolbe synthesis). Sodium acetate gives ethane, sodium propionate n-butane, calcium laurate, L, n-docosane, LI.



L LI

Of these general methods for decarboxylation only a few have been applied to the special case of the uronic acids.

(iii) The decarboxylation of uronic acids.

(a) General introduction.

The classical method of decarboxylation of uronic acids is that of Lefèvre and Tollens (61). It is based on the work of Mann (1894) and of Mann and Tollens (1896), who, using 12% hydrochloric acid as the decarboxylating reagent, quantitatively estimated glucuronic acid. The reaction was

formulated as follows:-

$$C_6H_{10}O_7 = C_5H_4O_2 + CO_2 + 3H_2O$$

Uronic acid Furfural

While the yield of CO_2 is quantitative, that of furfural has always been found to be less than theoretical, indicating that other complicating reactions take place as well as the decarboxylation.

Although hydrochloric acid is the best mineral acid for decarboxylation, other workers have used sulphuric acid (62), hydriodic acid (63), and phosphoric acid (64) (65).

Uronic acids have also been decarboxylated by heating; theoretical yields have been reported for glucuronic acid, galacturonic acid and alginic acid by heating at 255°C., the reaction being complete in 15 mins. (66).

The decarboxylation of uronic acids by amines in the presence of metal salts has been investigated (67); the corresponding pentose was isolated from the reaction products in small yield.

The decarboxylation of the dry silver salt of alginic acid, by treating a suspension of the salt in carbon tetrachloride with bromine, has been reported (68).

Various mechanisms for the decarboxylation reactions, both in aqueous mineral acid (69) (70) (71) and by metal salts in

amines (22) have been proposed and will be discussed later.

(b) The decarboxylation of uronic acids in aqueous mineral acid.

(i) Experimental methods used.

The original apparatus of Lefèvre and Tollens has been modified a large number of times. Table I gives a summary of the most important analytical features used by various workers.

Lefèvre and Tollens used 12% HCl as decarboxylating reagent. This appeared to be satisfactory until the introduction of 19% HCl (92) cut the time for complete decarboxylation from 4-5 hrs. to 1 ½-2 hrs.

Various authors (91) (86) maintain that the use of 19% HCl introduces errors by increasing the CO_2 produced from side reactions and from the decomposition of non-uronic acid materials. Other workers, however, reported (92) that there was very little difference in CO_2 produced from glucose when refluxed in 19% HCl or in 12% HCl.

The use of nitrogen as carrier gas is also of comparatively recent innovation (80) while CO_2-free air contains oxygen which is considered to add to the oxidation of non-uronic acid materials, so giving extraneous CO_2; some workers (82) report no significant difference in results obtained by using (a) air, (b) nitrogen.

TABLE I

Investigators	Decarboxylating agent	Carrier gas	Reaction Temp.	Reaction Time (hrs.)	Sample Wt. (mg.)	Scrubbers used	Method of CO_2 determination
Lefèvre, Tollens (1907) (61)	100 ml. 12% HCl	CO_2-free air	Reflux	3½	300	Water and $CaCl_2$	Gravimetric in Soda-lime
Nanji, Paton, Ling (1925) (74)	"	"	"	"	200-500	None	100 ml. N/10 Baryta
Ehrlich, Schubert (1929) (75)	"	"	"	8-10	500	Water and $CaCl_2$	Gravimetric in Soda-lime
Dickson, Otterson, Link (1930) (76)	"	"	"	5	200-500	$AgNo_3$ only	N/5 Baryta
Bowman, McKinnis (1930) (78)	200 ml. 12% HCl	"	"	3½-4	300	Mossy zinc	0.1N Baryta
Burkhart, Bauer, Link (1934) (79)	12% HCl satd. with NaCl	"	"	2-3	10	---	0.02N Baryta
Whistler, Martin Harris (1940) (80)	300 ml. 12% HCl	N_2 at 10 l./hr.	"	4	over 100	conc. H_2SO_4 P_2O_5, anhyd. $CuSO_4$	Soda-lime
Bartholomew, Norman (1941) (81)	12% HCl	CO_2-free air 2 l./hr.	135°C	5	---	---	0.1 N. NaOH

TABLE I (Continued)

Investigators	Decarboxylating agent	Carrier gas	Reaction Temp.	Reaction Time (hrs.)	Sample Wt. (mg.)	Scrubbers used	Method of CO_2 determination
Yackel, Kenyon (1942) (82)	12% HCl	CO_2-free air 100 bubble/min.	Reflux	8	50	Aniline-SO_4 conc. H_2SO_4	Gravimetric Soda-lime
Taylor, Fowler, McGee, Kenyon (1942) (83)	75 ml. 12% HCl	N_2 at 2 l./hr.	"	Up to 15	250	Aniline-SO_4 conc. H_2SO_4	"
Tracey (1948) (84)	0.25 ml. 12% HCl	Sealed tube	111°C	5	Up to 50	---	CO_2 measured manometrically
Browning (1949) (85)	12% HCl satd. with NaCl	N_2 at 1.7 l./hr.	Bath at 140°C	3-4	100	300ml. H_3PO_4 +10gm. Ag_2CO_3	Gravimetric Soda-lime
Vollmert (1949) (63)	12% HCl 20% HCl 57% HI	---	---	8 3-5 1-2	---	---	Gravimetric
Letzig (1950) (86)	100 ml. 12% HCl	CO_2-free air	Reflux	4	2-4gm. polysacc.	Water only Phloroglucinol	50ml. 0.1N. Baryta
Ogston, Stanier (1951) (87)	0.4 ml. 12% HCl	Sealed tube	110°C	5	0.2	---	0.2ml. 0.01N. $Ba(OH)_2$ Conway diffusion
Johansson, Lindberg, Theander (1954) (88)	25 ml. 12% HCl	Oxygen-free N_2 at 10ml./min.	Oil-bath 140°C.	3-4	10-75	Water	Titrimetric

TABLE I (Continued)

Investigators	Decarboxylating agent	Carrier gas	Reaction Temp.	Reaction Time (hrs.)	Sample Wt. (mg.)	Scrubbers used	Method of CO_2 determination
Buston (1932)(89)	13% HCl 90% satd. NaCl	CO_2-free air 4ml./min.	Reflux	70 min.	6-10	Ag_2SO_4 on glass-wool	10ml. 0.02N. $Ba(OH)_2$ Titre with $H_2\bar{O}x$
Lunde, Heen, Øy (1938) (90)	13% HCl 18% HCl	CO_2-free air	"	4 4	200	None	N/10 $Ba(OH)_2$
Maher (1946) (91)	50 ml. 12.5% HCl	"	150°C	3-4	12-15	"	0.04N. $Ba(OH)_2$
McCready, Swenson, Maclay (1946)(92)	19% HCl 12% HCl	"	Oil-bath 145°C.	1.5 5	250	Granulated Zn or Sn	0.25N. NaOH
Huber, Deuel (1951) (70)	40 ml. 20.24% HCl (const. boil.)	N_2 at 2 l./hr.	110°C 100°C	---	200	Phloroglucinol then $AgNO_3$ soln.	0.02N. Baryta
Barker, Foster, Siddiqui, Stacey (1958) (93)	3 ml. 19% HCl	N_2 at 1 bubble/min.	Oil-bath 145°C	2	20	None	5 ml. 0.25N. NaOH then $BaCl_2$, 0.1N. HCl titre
Meller (1954)(62)	27% w/w H_2SO_4	N_2 at 1.8-2 l./hr.	Reflux	6-7	200-500	Ag_3PO_4, cotton + resorcinol	Soda-lime
Anderson (1958) (94)	50 ml. 19% HCl	N_2 at 15ml./min.	110°C	2½	10-30	None	15ml. 0.05N. $Ba(OH)_2$

Whistler, Martin and Harris (80) used the very fast flow-rate of 10 litres per hour in a large apparatus, but Taylor, Fowler, McGee and Kenyon (83) found this too high to give reproducible results in kinetic runs where small variations in flow were unavoidable. Buston (89), using a modified Zeisel apparatus, used CO_2-free air at 4 ml./min., and modern workers tend to use flow-rates of 15-30 ml./min. or less.

19% HCl boils at 109°C. and 12% HCl at 105°C., yet oil-bath temperatures of 150°C. were used by most early workers. The replacement of the oil-bath by an electrothermal mantle designed for 50 ml. flasks (94) eliminates any charring on the walls of the 100 ml. reaction flasks; this decreases the evolution of spurious and non-reproducible quantities of CO_2, which are produced if heat is directly applied to a level higher than that of the refluxing liquid inside.

Various traps and scrubbers have been employed to remove traces of HCl carried over by the scavenging gas. Water (86) (88), water and calcium chloride (61) (75), mossy zinc (78), granulated zinc or tin (92), concentrated sulphuric acid (80) (82) (83), aniline salts (82) (83), silver nitrate (76) (70), other silver salts (85) (89), phloroglucinol (86), resorcinol and silver phosphate (62), or phloroglucinol and silver nitrate (70) have all been used as scrubbers.

Modern workers (101) (93) have found scrubbers unnecessary under certain conditions.

The CO_2 evolved has been measured manometrically (84), gravimetrically by absorption in soda-lime (83), and volumetrically by absorption in standard baryta (70) (94) or in sodium hydroxide (81) (92), the excess alkali being titrated with standard hydrochloric or oxalic acid (89), using phenolphthalein as indicator.

The tendency has been to decrease the apparatus and sample to the smallest size consistent with accurate determinations. Maher (91) found that traps were unnecessary if a long reflux condenser was used. The apparatus described in more recent papers has incorporated more efficient condensers (93) (94) and the necessity for traps, which made the older apparatus cumbersome and difficult to render leak-proof, has been eliminated.

(ii) Results obtained by previous workers.

Lefèvre and Tollens (61) applied their method of estimating uronic acids to derivatives of glucurone, and later authors (74) applied the method to pectins, gums and oxidised celluloses. Ehrlich and Schubert (75) found that pectic acid gave 20.00% CO_2 after 4 hrs. and 24.74% CO_2 after 8 hrs. in boiling 12% HCl (theoretical value 25.0%).

Link and co-workers (76) applied the method to pure galacturonic acid and glucurone as standards and obtained the theoretical yield. Various strengths of acid (95) were also used and it was found that under the conditions normally used for the

isolation of uronic acids, i.e. extraction with 2% HCl or 1N.H_2SO_4, decarboxylation of up to 5% of the uronic acid present takes place. Errors in the determination due to evolution of CO_2 from the non-uronic acid residues present in the polysaccharide materials were noted by various workers (79) (80).

Bowman and McKinnis (78) determined both the CO_2 and furfural yield from galacturonic acid, the furfural being steam-distilled during the reaction and estimated as the phloroglucide. No carbon monoxide was found to be liberated. The CO_2 yield was theoretical while that of furfural was only 34%.

Various authors found that the liberation of CO_2 from uronic acids was faster in strong acid than in weak, but no attempt to find an exact relationship was made (80) (95) (65).

Letzig (86) commented adversely on the use of 19% HCl; he suggested that the increased formation of CO_2 from side-reactions outweighs the saving in time gained by the use of this strength of acid. His investigation covered a very wide range of natural compounds.

McCready, Swenson and Maclay (92), using both 12% and 19% HCl as the decarboxylating reagent, compared the results and concluded that there was no advantage in using 12%; moreover, the theoretical yield of CO_2 from alginic acid could only be obtained using 19% HCl. They also found that approximately equal amounts of CO_2 were liberated from glucono-δ-lactone, oxalic acid, mucic acid, starch and sucrose, but this has been

disproved by later workers (70).

The first investigation of the kinetics of the decarboxylation of uronic acids in 12% HCl was carried out by Taylor, Fowler, McGee and Kenyon (83) who calculated the first order rate constants by applying the method of Guggenheim (96). These authors also estimated the total CO_2 given off in 24 hrs. from various other carbohydrate materials including gluconic acid and oxalic acid (8% and 5% respectively). Table II shows the results obtained.

TABLE II

Rate constants for the decarboxylation of various uronic acids in 12% HCl

Substance	k	
	$hr.^{-1}$	$sec.^{-1}$
L-ascorbic acid	1.70	4.72×10^{-4}
D-galacturonic acid monohydrate	0.783	2.18×10^{-4}
D-glucuronic acid	0.457	1.27×10^{-4}
Alginic acid	0.322	8.94×10^{-5}

Their results showed that ascorbic acid decarboxylates more than twice as fast as galacturonic acid. This, they proposed, was due to the effect of the enediol structure on the stability of the adjacent lactonised carboxyl group.

Huber and Deuel (70) investigated the rates of decarboxylation of various uronic acids in constant boiling hydrochloric acid (20.24%($^{w}/_{w}$) HCl) at 110°C. and 100°C., assuming a first order reaction. The rate constants found are shown in Table III.

TABLE III

Rate constants for the decarboxylation of various uronic acids in 20.24% HCl at 110°C.

Substance	k sec.$^{-1}$
D-galacturonic acid monohydrate	$2.187 \cdot 10^{-4}$
D-galacturonic acid naphthoresorcinol derivative	$2.753 \cdot 10^{-5}$
Polygalacturonic acid	$2.160 \cdot 10^{-4}$
Methyl ester of 2,3,4,trimethyl methyl-galacturonoside	$3.309 \cdot 10^{-5}$
2-D-galacturonido-L-rhamnose	$2.795 \cdot 10^{-4}$
Methyl-ester of pentamethyl-2-D-galacturonido-methyl-L-rhamnoside	$3.875 \cdot 10^{-5}$
Mucic acid	$4.796 \cdot 10^{-5}$
D-mannuronic acid	$1.670 \cdot 10^{-4}$
Alginic acid	$1.466 \cdot 10^{-4}$
D-glucuronic acid lactone	$1.648 \cdot 10^{-4}$
D-gluconic acid lactone	$5.412 \cdot 10^{-5}$
Saccharic acid	$2.517 \cdot 10^{-5}$
Lactic acid	$3.989 \cdot 10^{-6}$

The value obtained for galacturonic acid is the same as found by Taylor, Fowler, McGee and Kenyon (see Table II) despite the fact that they are in very different concentrations of acid. This, therefore, throws some doubt on the validity of these results.

The Arrhenius activation energies for the decarboxylation of galacturonic acid and mannuronic acid were calculated from the rate constants at 100° and 110°C., the values being 37 and 43 kcals. respectively.

Huber and Deuel postulated a mechanism for the decarboxylation reaction based on the differences in rate between ring and non-ring acids. The rate constants for the decarboxylation of sugars were also calculated assuming a zero order reaction. As can be seen from Table IiI the rate constants for the decarboxylation of mucic acid, gluconic acid lactone, saccharic acid and lactic acid were also calculated as first order constants although the values of k from which the mean rate constants were calculated showed considerable drift.

Conrad (100) stated that sulphuric acid was inferior to hydrochloric acid in the decarboxylation of uronic acids. However, Meller (62) investigated the decarboxylation of uronic acid materials in 24-27% sulphuric acid and obtained theoretical yields of CO_2 from glucurone in 6-7 hrs. The uronic acid content of polysaccharides was obtained by substituting the CO_2 values in an equation derived from consideration of two consecutive first order reactions, the rate constants previously found for the pure

uronic acids being substituted in the derived expression. Since this method requires a prior knowledge of the uronic acids present in a polysaccharide, it appears unlikely that it will be widely adopted for the determination of the uronic acid content of polysaccharides.

In 1948, Tracey (84) applied a new technique to the determination of uronic acids. The samples were heated in a sealed tube with 12% HCl for 5 hrs. and the CO_2 was estimated manometrically. This method was applied to a large variety of sugars and related compounds; the values obtained were compared with those of other workers. Ogston and Stanier (87) modified this method for use with 0.2 mg. quantities of uronic acid, estimating the CO_2 by a Conway diffusion technique (97). It was stated that the results agreed with those obtained by Tracey.

The flow apparatus can also be modified to the micro scale. Buston (89) used a modified Zeisel apparatus with some success for the determination of uronic acids in pectins. Two designs of apparatus for use on the semi-micro scale were described recently (93) (94).

Stutz and Deuel (65) using the method of Ogston and Stanier (87) decarboxylated galacturonic acid in HCl, H_2SO_4, and H_3PO_4 and compared the molar yields of furfural and reductic acid with that of CO_2. The results for L-arabinose were also given for comparison.

TABLE IV

The effect of mineral acid on various carbohydrate materials

Substance	Mineral acid and concentration	Moles/mole.		
		CO_2	Furfural	Reductic acid
D-galacturonic acid monohydrate	3.5N.HCl for 4 hrs.	1.0	0.32	0.19
	3.5N.H_2SO_4 for 4 hrs.	0.55	0.07	0.25
	3.5N.H_3PO_4 for 4 hrs.	0.12	0.01	0.07
	3.5N.H_3PO_4 for 8 hrs.	0.23	0.02	0.14
L-arabinose	3.5N.H_3PO_4 for 4 hrs.	0.0	0	0
	3.5N.H_3PO_4 for 8 hrs.	0.01	0	0

They also compared the amount of CO_2 liberated from various uronic acids by 1.75 N.HCl in 4 hrs. and 0.01N.HCl in 20 hrs. They considered that an α-β-unsaturated carboxylic acid intermediate was unlikely and postulated a β-γ-unsaturated carboxylic acid intermediate. They put forward a mechanism for the decarboxylation, arriving at the conclusion that one of the dehydration steps is slower than the CO_2 elimination and is therefore rate determining. This also explained the small reductic acid yield from pentoses. A similar mechanism was proposed by Isbell (69) in 1944; all these mechanisms will be discussed later.

The work reported in this thesis was undertaken (1) to find the best analytical procedure for the determination of uronic acids, and (2) to find the rate of decarboxylation of various uronic acids, and to compare the rate constants with those previously determined.

(iv) Experimental.

The apparatus used was fully described by Anderson (94).

The apparatus is shown in Fig. I. The ether-type condenser has two concentric coils wound closely round a central vertical conductor. The condenser jacket is virtually filled with cooling coils, and the internal volume of the condenser and trap is only 150 ml. Nitrogen is the scavenging gas at a flow-rate of 15 ml. per minute, this passing through "Sofnolite" to remove carbon dioxide before entering the apparatus. The titrimetric method is used, this being the most convenient for routine purposes. The absorption-trap is constructed from a B34 cone and socket. About 50% of the ground-glass cone is cut off; this permits closer fit of the baffle plates inside the tube. The baffles are made either of glass or of thin polythene discs fitted on to the delivery tube, which ends in an approx. 1 cm. diameter sintered disc. The space left between this disc and the bottom of the tube allows the coagulated $BaCO_3$ precipitate to settle. Grade 1 porosity is most suitable; a fine stream of bubbles is given with very little back pressure. For kinetic measurements a two-way junction stop-cock

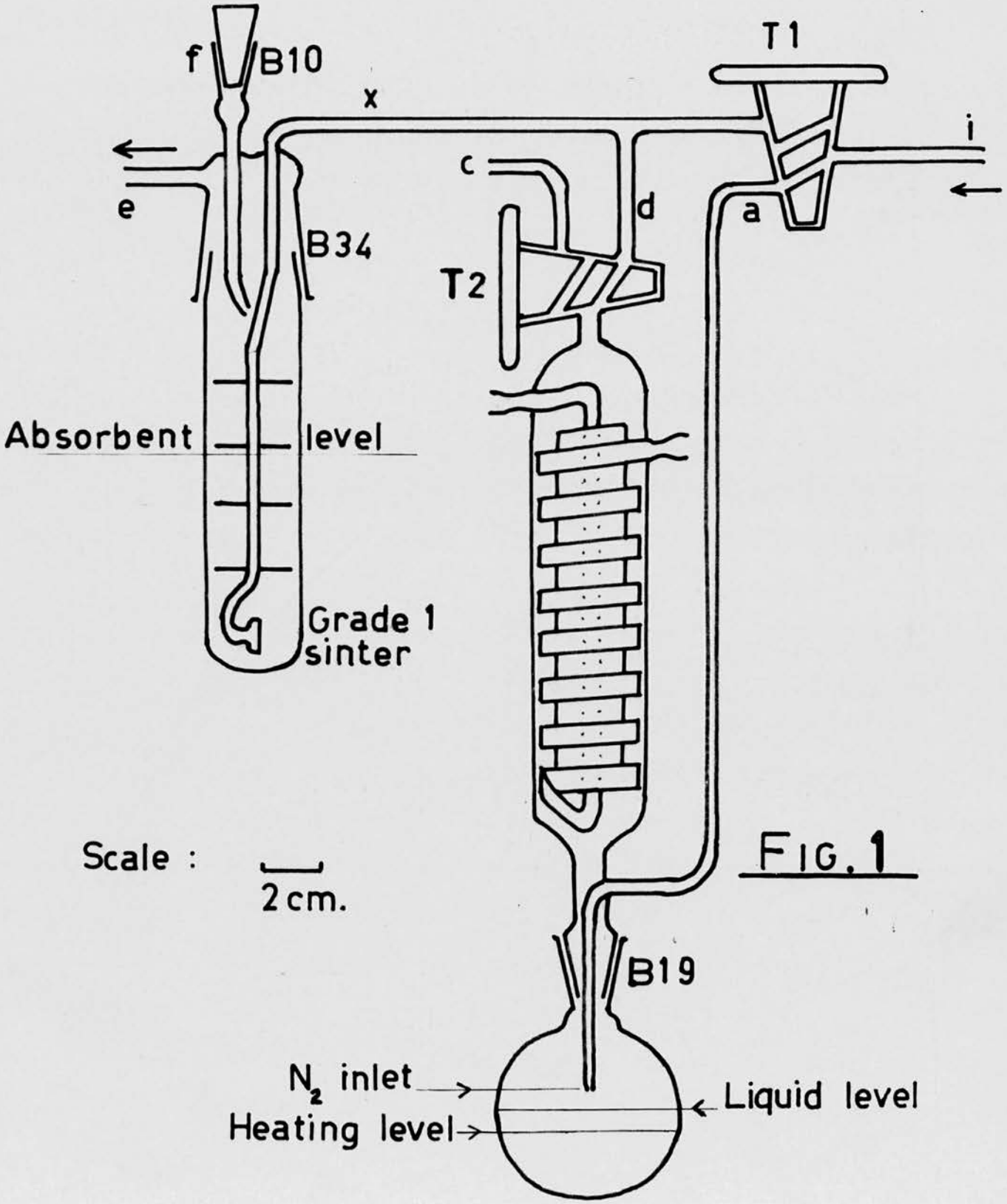

f
B10
x
T1
i
c
d
a
e
B34
T2
Absorbent
level
Grade 1
sinter
Scale :
2 cm.
B19
N_2 inlet
Liquid level
Heating level

FIG. 1

system is introduced at x in Fig. I, so that the gas stream can be switched through different traps. A pair of traps fitted with closely matching sintered discs is used, so avoiding differing back pressures, which affect the reflux temperature, and hence the reaction rates. The decarboxylating reagent is 19%($^{w}/_{w}$) hydrochloric acid. The apparatus has no blank under normal working conditions.

Initially pumice impregnated with copper sulphate was used as a scrubber to remove any trace of hydrochloric acid, but work with carbon-14 indicated that this substance absorbed some of the CO_2 and then desorbed it slowly. This tended to give a trail of CO_2 which gave incorrect kinetics of the decarboxylation, and so its use was discontinued.

The trap for CO_2 absorption was found to be very efficient; when a similar trap was fitted to the exit of the first, and a flow-rate of 30 ml./min. (i.e. double that normally used) was employed, no titratable CO_2 was detected in the second cell. Similarly, in a 24 hr. run, replacement of this second cell by a trap immersed in liquid oxygen and analysis of the contents by infra-red spectroscopy showed that all CO_2 had been removed by the first absorption trap.

The 100 ml. reaction flask containing 50 ml. of 19% ($^{w}/_{w}$) HCl is heated on an electrothermal mantle, Type M101, designed for 50 ml. flasks. The heat available is quite adequate to boil the acid in the flask, and the level of heating avoids charring on the walls of the reaction flask.

A special flask fitted with a side pocket containing mercury and a thermometer allowed the temperature of the reaction mixture to be obtained.

At the start of an analysis, 25 ml. of distilled water are put into the trap and nitrogen allowed to scavenge the apparatus for 20 min.; the water is then, if necessary, made neutral to phenolphthalein, by adding, through f, the required amount of 0.05N.$Ba(OH)_2$.

The apparatus is permanently held in clamps round the B34 socket of the absorption cell and round the condenser. The pressure-stabilised flow of pure nitrogen (oxygen-free grade), regulated by needle-valves and rotameter to 15 ml./min., enters the apparatus at i. The sample of material is weighed out in a small glass bucket and placed in the flask; 50 ml. of 19% HCl(w/w) is added and the flask then placed in position, a trace of silicone grease being used to give a good joint. In all the quantitative work, long-necked flasks with B19 joints having "compo" tubing tightly coiled round the neck were used. Water flowing through this tubing cooled the joint and decreased the chance of any leak occurring as a result of the silicone grease softening and "running".

T_1 and T_2 are positioned so that nitrogen flows through d. 10 ml. of 0.05N.baryta are added to the trap through f from a conventional CO_2-free storage system, and the tube f washed down with distilled water until the absorbent level is just below

the third baffle. The first traces of CO_2 reach the trap 10 mins. after the liquid in the flask begins to boil. The third and fourth baffles act as an anti-spray device, and the first and second create a turbulent mixing action inside the absorption trap.

The gas is collected from the time the heater is switched on until the end of the reaction. This ensures that any CO_2 which is liberated at a temperature below the boiling-point is absorbed. The time of reaction is taken from when the gas first arrives in the trap, which is 10 min. after the liquid boils.

When decarboxylation is complete, tap T_1 is turned so that the nitrogen flows through b and through the trap. T_2 is turned so that the slight pressure in the flask is released through c. The heating mantle is switched off and the excess baryta in the trap carefully back-titrated with standard 0.02N. HCl. This titration is done slowly to allow adequate mixing inside the trap; whilst it is in progress, the second flask with a new sample is placed in position and by turning T_1 the nitrogen is passed through a to c. The absorption trap is then lowered, thoroughly rinsed, and replaced. Thus the double two-way tap system permits rapid duplication of determinations.

Reagents and chemicals.

The D-galacturonic acid used in the following determinations was supplied by Roche Biochemicals Ltd., and was chromatographically pure (only a faint trace of D-galactose was detected). Preliminary analyses showed that this material was the purest available from a number of commercial sources. Glucurone, supplied by Roche Biochemicals Ltd., was used after several recrystallisations from water. Elemental analyses were carried out by Weiler and Straus, Microanalytical Lab., Oxford (for galacturonic acid) and by Dr. R.A. Chalmers and Miss D.A. Thomson, Dept. of Chemistry, Aberdeen University (for glucurone).

Glucurone:- Found: C, 40.8%, 40.7%; H, 4.6%, 4.6%;
Theoretical: C, 40.91%; H, 4.58%.

Galacturonic acid (as monohydrate):-
Found: C, 34.84%; H, 5.69%.
Theoretical: C, 33.97%; H, 5.70%.

The neutralisation equivalents were also determined by adding excess standard NaOH to a known weight of uronic acid sample and titrating the excess with standard HCl (after 10 min. in the case of monomers, but 2 hrs. in the case of alginic acid, i.e. when it had all dissolved).

	% purity (by neutralisation equivalent)
D(+)Glucurone	97.2
D(+)Galacturonic acid monohydrate	96.0
Alginic acid	97.1

The alginic acid, specially prepared by methods designed to avoid degradation, was obtained from Dr. G.O. Aspinall of this department. These substances were, therefore, taken as standards in the following work.

The trigalacturonic acid (found to be chromatographically free from mono-, di- and trigalacturonic acids but containing a little poly galacturonic acid) and the keto aldonic acids used in this part were obtained from Dr. W.W. Reid, Dept. of Chemistry, University of Bristol.

The L-ascorbic acid was obtained from British Drug Houses Ltd.

EXPERIMENTAL RESULTS

(i) Investigation of the order of the decarboxylation reaction.

The kinetics of the decarboxylation of uronic acids have been investigated by previous workers (70) (83). They assumed that the liberation of CO_2 followed, to a close approximation, simple first order kinetics and on this basis calculated rate constants.

For a first order reaction the rate of decarboxylation should be proportional to the concentration of uronic acid.

$$\text{i.e. } \frac{d[CO_2]}{dt} = k_1[\text{uronic acid}] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

Hence, plotting $\frac{[CO_2]}{[\text{uronic acid}]}$ versus time gives a typical exponential curve, of the type shown in Fig. 2, from which k_1 can be calculated.

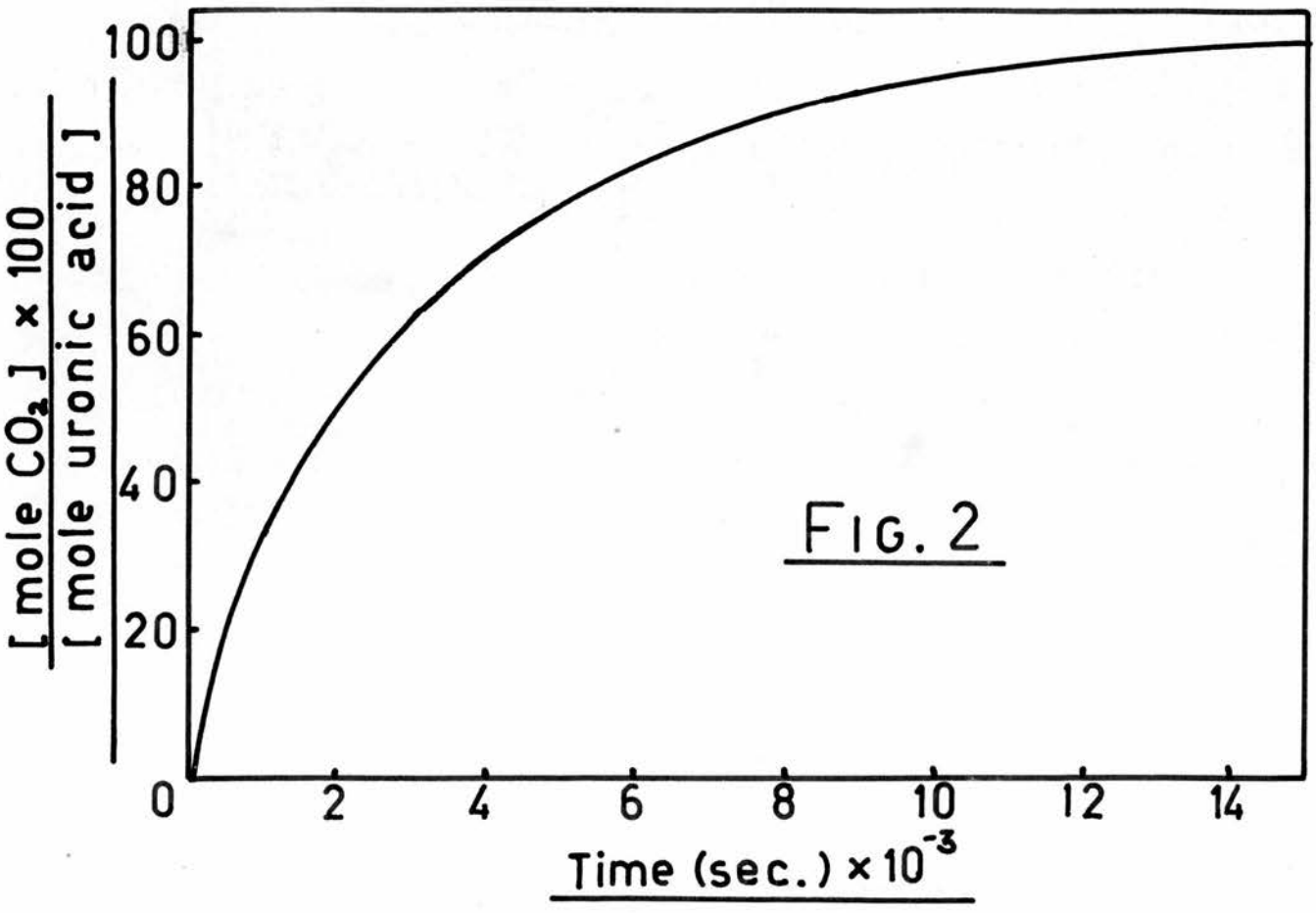


FIG. 2

A plot of this function against time should also give the same curve regardless of the weight of uronic acid taken.

The results in this section are all expressed as percentage moles CO_2 per mole of uronic acid.

Preliminary experiments showed that 3.8% (w/w) hydrochloric acid was a suitable strength of acid to use in this experiment, in which it was desirable to use sample weights ranging from 30 mg. - 1 g.

Table V(a) shows the results obtained when different weights of D-galacturonic acid monohydrate were decarboxylated, using nitrogen as flow-gas.

TABLE V(a)

The decarboxylation of various weights of D-galacturonic acid monohydrate in boiling 3.8% (w/w) hydrochloric acid.

Time (sec.)	% moles CO_2 per mole uronic acid		
	Wt. of uronic acid samples taken		
	30.0 mg.	322.25 mg.	999.31 mg.
2,000	7.9	8.0	8.2
4,000	15.0	15.2	15.7
6,000	21.0	21.3	22.0
10,000	32.5	33.1	34.0
15,000	46.2	46.7	48.1
20,000	57.4	58.3	60.1
40,000	82.2	83.5	86.1
60,000	94.0	95.3	98.2
80,000	98.2	99.8	102.9

Inspection of the results given in Table V(a) shows that for 30 mg., 322 mg., and 999 mg. samples of uronic acid, the molar percentage release of carbon dioxide was substantially independent of sample weight. The figures quoted are the average values obtained from several determinations for each of the sample weights at each of the times shown. The small non-linear increase in molar yield of CO_2 with increasing sample weight may be due either to experimental error or to increasing contributions in the amount of CO_2 produced in minor side reactions.

The decarboxylation reaction is therefore of first order, and subsequent work with other uronic acid substrates indicated that the results shown above for galacturonic acid were quite typical.

From the data presented in Table V(a), the rate constant was found by Guggenheim's method (96), which is useful in that a knowledge of the exact purity of the uronic acid sample is not required. The mathematical treatment is as follows:-

The reaction may be formulated:-

$$\text{Uronic acid} \xrightarrow{k_1} CO_2 + \text{product} \ldots\ldots \text{(fast)} \ldots\ldots\ldots\ldots (2)$$

$$\text{i.e.} \quad A \xrightarrow{k_1} C_1 + B \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

$$\text{Product} \xrightarrow{k_2} CO_2 + \text{products} \ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

where the CO_2 in (4) is produced by side-reaction.

If A_o = initial concentration of uronic acid

A = concentration of uronic acid at time t

C_1 = amount of CO_2 formed in time t

Then $\frac{dA}{dt} = -k_1 A$, i.e. $A = A_o e^{-k_1 t}$ ..... (5)

Now $C_1 = A_o - A_o e^{-k_1 t}$, i.e. $A_o - C_1 = A_o e^{-k_1 t}$ ..... (6)

Consider the values of the total CO_2, C_t at times t_1 and t_2. The equations then become

$$A_o - C_{t_1} = A_o e^{-k_1 t_1} \quad \text{..... (7)}$$

$$A_o - C_{t_2} = A_o e^{-k_1 t_2} \quad \text{..... (8)}$$

Subtracting (8) from (7)

$$-C_{t_1} + C_{t_2} = A_o(e^{-k_1 t_1} - e^{-k_1 t_2}) \quad \text{..... (9)}$$

If $t_2 - t_1 = \Delta$

Then $C_{t_{1+\Delta}} - C_{t_1} = A_o e^{-k_1 t_1}(1 - e^{-k_1 \Delta})$ ..... (10)

So a plot of $\log_{10}(C_{t_{1+\Delta}} - C_{t_1})$ versus t_1 should give a straight line with slope $2.303\,k_1$ provided Δ is 2-3 times the half period of the reaction.

From the data shown in Table V(a) we can construct Table V(b).

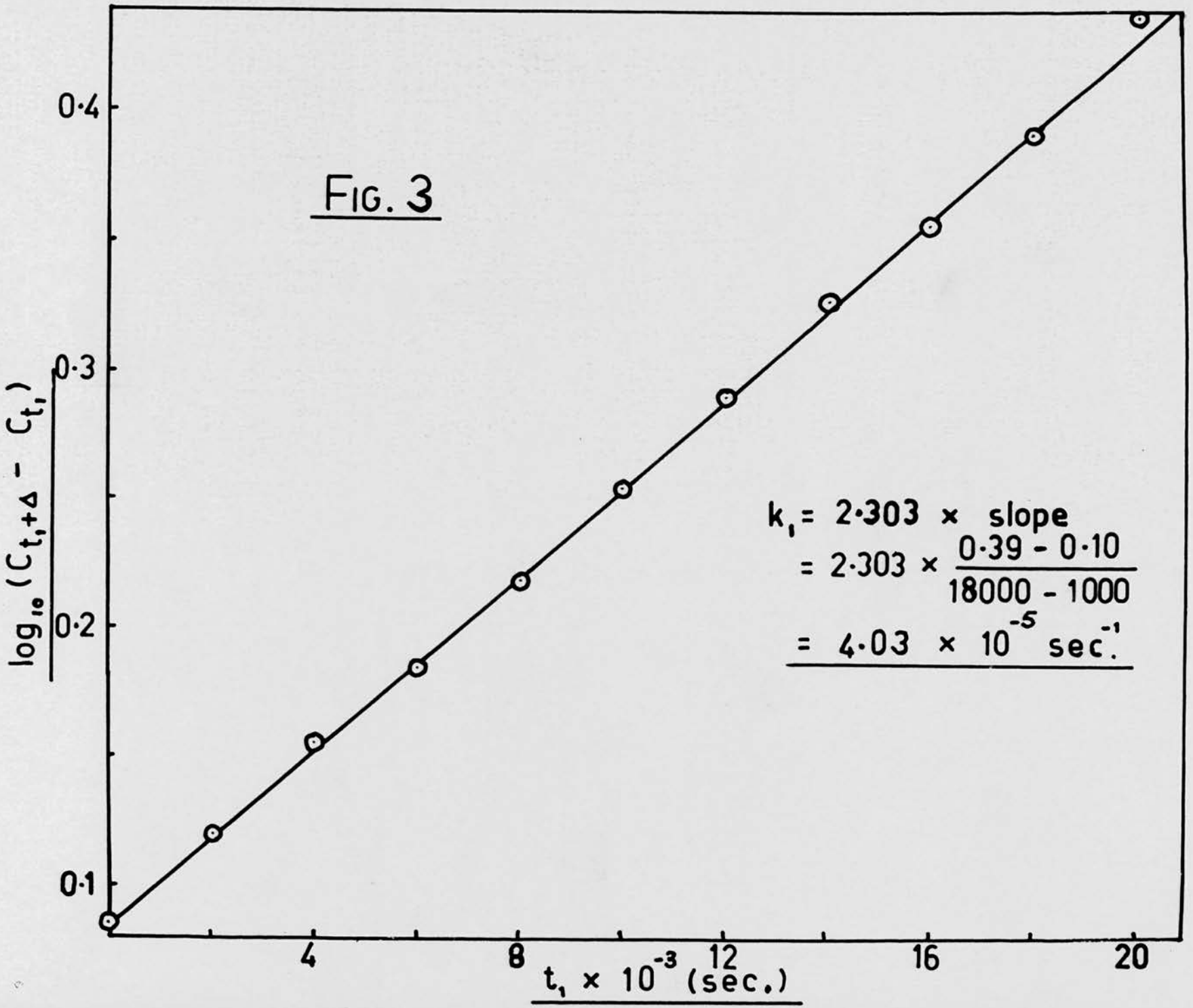


FIG. 3
0·4
0·3
0·2
0·1
$\log_{10}(C_{t_1+\Delta} - C_{t_1})$
4
8
12
16
20
$t_1 \times 10^{-3}$ (sec.)
$k_1 = 2{\cdot}303 \times \text{slope}$
$= 2{\cdot}303 \times \frac{0{\cdot}39 - 0{\cdot}10}{18000 - 1000}$
$= 4{\cdot}03 \times 10^{-5}\ \text{sec.}^{-1}$

TABLE V(b)

Example of the use of Guggenheim's method for the calculation of k_1 for the decarboxylation of D-galacturonic acid monohydrate in 3.8% (w/w) HCl.

Δ = 40,000 sec.

t_1	$t_1 + \Delta$	C_{t_1}	$C_{t_1+\Delta}$	$C_{t_1+\Delta} - C_{t_1}$	$\log_{10}(C_{t_1+\Delta} - C_{t_1})$	
0	40000	0	0.822	0.822	$\bar{1}$.9149	-0.0851
2000	42000	0.079	0.838	0.759	$\bar{1}$.8802	-0.1198
4000	44000	0.150	0.851	0.701	$\bar{1}$.8457	-0.1543
6000	46000	0.210	0.865	0.655	$\bar{1}$.8162	-0.1838
8000	48000	0.270	0.878	0.608	$\bar{1}$.7839	-0.2161
10000	50000	0.325	0.883	0.558	$\bar{1}$.7466	-0.2534
12000	52000	0.375	0.890	0.515	$\bar{1}$.7118	-0.2882
14000	54000	0.441	0.913	0.472	$\bar{1}$.6739	-0.3261
16000	56000	0.482	0.923	0.441	$\bar{1}$.6444	-0.3556
18000	58000	0.530	0.932	0.406	$\bar{1}$.6085	-0.3915
20000	60000	0.574	0.940	0.366	$\bar{1}$.5635	-0.4365

From the data given in Table V(b) the graph of $\log_{10}(C_{t_1+\Delta} - C_{t_1})$ versus t_1 shown in Fig.3 was drawn, and the value of $k_1 = 4.03 \times 10^{-5} sec^{-1}$ calculated as shown.

Results were obtained in similar fashion for other acids, and these are presented along with the appropriate tables of results in later parts of this thesis.

(ii) Comparison of the properties of nitrogen, oxygen and hydrogen as scrubbing gases.

In the analytical determination of uronic acids it is customary to use a continuous flow-gas scavenging system. If CO_2-free air is used, the continuous replacement of oxygen in the reaction-flask, even at comparatively low flow-rates, could conceivably lead to overoxidation and to undesirable production of CO_2 in side-reactions. Table I (p.33) indicates that some authors used cylinder nitrogen in place of CO_2-free air.

In order to test whether the presence of oxygen did in fact introduce errors, a quantitative study was carried out using 19% (w/w) HCl on samples (30 mg.) of D-galacturonic acid monohydrate. The results are given in Table VI(a): each molar yield quoted is the average obtained from several determinations made, each on 30 mg. samples, at the reaction times stated. For instance, using nitrogen, the molar yields of CO_2 from three successive 30 mg. samples were, after 9000 secs., 96.3, 95.6, 96.1% respectively. It is therefore considered that the differences in the results obtained for nitrogen, oxygen and hydrogen are real, and are not due to experimental error.

The rate constants were calculated by Guggenheim's method (96) from the data given in Table VI(a). In 19% (w/w) HCL, the decarboxylation reaction is so rapid that it is difficult to obtain accurate and reproducible molar yields of CO_2 for time intervals less than about 600 secs.

TABLE VI(a)

A comparison of the rate of decarboxylation of D-galacturonic acid monohydrate in boiling 19% HCl (w/w) using oxygen, nitrogen and hydrogen as flow gases.

Time (sec.)	% moles CO_2 per mole uronic acid		
	Flow-gas		
	Nitrogen	Oxygen	Hydrogen
1000	67.3	60.1	69.9
2000	86.0	79.0	80.0
3000	91.2	85.5	84.2
4000	93.0	88.6	86.6
9000	96.0	95.0	90.9
15000	98.5	97.5	92.7
20000	99.8	99.6	93.8
40000	103.0	105.1	97.6
60000	105.8	110.1	100.7
80000	108.0	115.1	103.4
$k_1(sec^{-1})$	1.2×10^{-3}	9.8×10^{-4}	1.7×10^{-3}

The values of k_1 were obtained by calculation based on the initial part of the curve.

From the data it is seen that the rate of the initial reaction is in the order

Hydrogen > nitrogen > oxygen

but the final yield of CO_2 after about 24 hrs. (86400 sec.) is in the order

Oxygen > nitrogen > hydrogen.

It would therefore appear that hydrogen is fundamentally preferable to oxygen or nitrogen, giving more rapid initial decarboxylation and less CO_2 from side reactions. The percentage purity of the galacturonic acid sample was earlier found from the determination of the neutralisation equivalent to be 96.0. From Table VI(a) it is seen that this value is only reached after approximately 35000 sec. when hydrogen is the flow-gas, while with nitrogen the time required is 9000 sec., and with oxygen slightly more. It is obvious that the kinetics vary significantly with the flow-gas used.

Considering all the factors involved, nitrogen is preferable as flow-gas in routine analyses, and, in order to ensure constancy of experimental conditions, it was used in all subsequent investigations.

(iii) <u>The determination of Arrhenius activation energies and frequency factors for the decarboxylation reaction of uronic acids and related compounds</u>.

The quantities which are usually computed from measurements of temperature dependence of reaction rates are the parameters A and E_A of the Arrhenius equation

$$k_1 = Ae^{-E_A/RT} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (11)$$

where E_A is the Arrhenius activation energy

A is the Arrhenius frequency factor

R is the gas constant

and T the absolute temperature.

E_A approximately equals the activation energy, and log A is an approximate measure of the entropy of activation. This relationship is obtained by comparison of equation (11) with that obtained from transition state theory, and is true for a series of similar reactions.

The structural differences between certain uronic acids can be considerable; apart from differing internal configuration of hydroxyl groups, glucurone has two five membered rings, galacturonic acid is a pyranose with one molecule of water of crystallisation, trigalacturonic acid is a trimer of galacturonic acid and alginic acid is a polyuronide. Such differences, it was considered at this stage, might significantly affect the values of the activation energy and A factor.

The values of k_1 at five selected temperatures, e.g. 90°, 95°, 100°, 105°, and 112°C., were therefore determined by finding the molar yields of CO_2 given on decarboxylation of 30 mg. samples in 19% (w/w) HCl by the following substances, the data obtained being given in the Tables indicated:-

D(+)Glucurone	Table VII(a)
D(+)Galacturonic acid monohydrate	" VII(b)
Trigalacturonic acid	" VII(d)
Alginic acid	" VII(e)
Ca-5-keto-D-gluconate	" VII(f)
2-keto-L-galactonic acid	" VII(g)
Ca-2-keto-D-gluconate	" VII(h)
L-ascorbic acid	" VII(j).

In addition, similar experiments were carried out in 3.8% (w/w) HCl on galacturonic acid monohydrate: Table VII(c). This was done since 3.8% HCl had been used to prove the kinetics with respect to uronic acid concentration (see Table VI(a), p.56); it seemed desirable to find if any difference in mechanism resulted from changing the HCl concentration to 19% HCl. Consequently, the activation energy for the reaction in 3.8% HCl was found. The values (from Table VI(a)) compare favourably with those for 19% HCl.

It was observed during a preliminary investigation that some keto-aldonic acids gave first order kinetic curves and rates of reaction similar to those of uronic acids. It was considered that, if the calculated E_A and A factors were also similar to those of uronic acids, then an analogous decarboxylation mechanism may be operative. If this were so, a useful guide to formulation of the actual reaction mechanism would be established.

The values of k_1 for Ca-5-keto-D-gluconate (Table VII(f))

indicate a very rapid decarboxylation reaction. This leads to greater errors in the values of k than is the case with slower reactions. Using the data shown in Tables VII(a)-(j), the values of E_A are found from a plot of $\log_{10} k_1$ versus $(\frac{1}{T})$; the slope of the line obtained is $-\frac{E}{2.303R}$, and hence

$$\log_{10} A = \log_{10} k_1 + \frac{E}{2.303R} \cdot \frac{1}{T} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (12)$$

TABLE VII(a)

The temperature dependence of the rate of decarboxylation of D(+)glucurone in 19% (w/w) hydrochloric acid.

Time (sec.)	% uronic acid (% moles CO_2 per mole uronic acid)				
	112°C	105°C	100°C	95°C	90°C
1000	52.5	27.6	17.5	13.7	7.8
2000	74.9	48.8	34.3	26.7	14.7
4000	90.0	72.6	60.0	47.6	28.8
6000	94.8	83.7	75.6	62.0	41.8
9000	97.0	87.9	85.9	77.0	58.1
12000	98.0	94.5	90.5	84.5	68.2
15000	98.8	96.5	93.1	89.2	76.5
20000	99.7	97.9	95.7	93.1	84.9
30000	101.8	100.8	99.4	97.5	93.2
40000	103.8	103.1	101.9	99.9	97.2
60000	106.5	105.8	104.8	103.6	101.3
80000	107.6	107.1	106.4	105.0	102.7
$k_1(sec^{-1})$	6.5×10^{-4}	3.7×10^{-4}	2.6×10^{-4}	1.6×10^{-4}	1.0×10^{-4}
$\log_{10} k_1$	-3.19	-3.42	-3.59	-3.79	-3.99
$\frac{1}{T}\times10^3$	2.597	2.646	2.681	2.717	2.755

TABLE VII(b)

The temperature dependence of the rate of decarboxylation of D-galacturonic acid monohydrate in 19% (w/w) hydrochloric acid.

Time (sec.)	% uronic acid (% moles CO_2 per mole uronic acid)				
	112°C	105°C	100°C	95°C	91°C
1000	67.3	36.5	21.6	16.6	11.0
2000	86.0	58.4	40.5	30.6	21.4
4000	93.0	81.5	67.1	53.3	39.5
6000	94.9	89.6	80.1	70.2	53.3
9000	96.0	92.3	86.8	83.1	69.6
10000	96.8	93.9	88.2	85.0	74.5
15000	98.2	96.4	92.7	90.7	87.5
20000	99.6	98.6	96.0	94.3	92.8
40000	103.0	103.5	103.6	103.1	102.6
60000	105.8	106.4	107.2	107.5	107.7
80000	108.0	108.5	109.2	110.0	110.8
$k_1(sec^{-1})$	1.2×10^{-3}	5.7×10^{-4}	3.6×10^{-4}	2.2×10^{-4}	1.3×10^{-4}
$\log_{10} k_1$	-2.94	-3.24	-3.45	-3.66	-3.88
$\frac{1}{T} \times 10^3$	2.597	2.646	2.681	2.717	2.747

TABLE VII(c)

The temperature dependence of the rate of decarboxylation of D-galacturonic acid monohydrate in 3.8% (w/w) hydrochloric acid.

Time (sec.)	% uronic acid (% moles CO_2 per mole uronic acid)		
	103°C	100°C	90°C
2000	7.9	6.1	3.7
4000	15.0	12.0	6.4
6000	21.0	17.8	8.5
10000	32.5	28.9	13.0
15000	46.2	41.0	18.0
20000	57.4	50.4	22.9
30000	72.3	63.5	31.0
40000	82.2	74.0	38.5
60000	94.0	88.4	50.6
80000	98.2	96.1	60.6
$k_1(sec^{-1})$	4.0×10^{-5}	3.1×10^{-5}	1.2×10^{-5}
$\log_{10} k_1$	-4.39	-4.51	-4.92
$\frac{1}{T} \times 10^3$	2.660	2.681	2.755

TABLE VII(d)

The temperature dependence of the rate of decarboxylation of trigalacturonic acid in 19% (w/w) hydrochloric acid.

Time (sec.)	% uronic acid (% moles CO_2 per mole uronic acid)				
	112°C	105°C	100°C	95°C	90°C
1000	73.0	39.9	25.0	16.3	10.2
2000	85.1	64.4	44.2	32.6	20.6
4000	90.2	84.3	70.3	59.6	41.0
6000	92.5	88.2	80.8	72.5	56.8
10000	95.1	92.0	87.5	82.4	75.2
14000	96.6	94.1	89.6	87.0	83.2
16000	97.7	95.1	91.0	88.6	85.2
20000	99.2	96.6	93.0	91.0	88.4
40000	104.9	102.3	98.2	97.3	95.7
60000	108.1	103.7	102.3	100.2	99.0
80000	110.0	106.8	104.0	101.7	101.1
$k_1(sec^{-1})$	1.3×10^{-3}	6.6×10^{-4}	3.8×10^{-4}	2.8×10^{-4}	1.7×10^{-4}
$\log_{10}k_1$	-2.88	-3.18	-3.41	-3.55	-3.78
$\frac{1}{T}\times10^3$	2.597	2.646	2.681	2.717	2.755

TABLE VII(e)

The temperature dependence of the rate of decarboxylation of alginic acid in 19% (w/w) hydrochloric acid.

Time (sec.)	% uronic acid (% moles CO_2 per mole uronic acid				
	112°C	105°C	100°C	95°C	90°C
1000	33.9	17.8	11.2	8.8	3.9
2000	59.0	35.2	22.9	17.0	10.2
4000	82.8	62.5	43.8	32.1	19.4
6000	91.2	79.1	60.8	45.3	27.8
9000	97.1	87.8	75.9	60.8	39.1
12000	99.5	92.9	85.2	69.6	48.2
15000	101.1	96.3	90.5	76.5	55.4
20000	103.0	100.0	96.1	85.6	66.0
40000	107.7	106.3	104.3	102.2	90.8
60000	110.0	109.0	108.0	107.0	101.2
80000	111.0	110.1	109.3	108.3	105.3
k_1(sec.$^{-1}$)	4.6×10^{-4}	2.4×10^{-4}	1.5×10^{-4}	9.9×10^{-5}	5.7×10^{-5}
log k_1	-3.33	-3.62	-3.81	-4.02	-4.25
$\frac{1}{T}\times10^{3}$	2.597	2.646	2.681	2.717	2.755

TABLE VII(f)

The temperature dependence of the rate of decarboxylation of Ca-5-keto-D-gluconate in 19% (w/w) hydrochloric acid.

Time (sec.)	% moles CO_2 per mole				
	112°C	105°C	100°C	95°C	90°C
500	75.0	57.9	34.8	28.0	20.8
1000	83.5	74.9	57.7	47.8	38.2
2000	87.1	83.0	76.1	72.2	63.6
3000	89.4	86.2	83.5	81.2	77.2
4000	89.4	88.1	86.7	85.5	82.1
6000	90.9	90.3	88.6	87.9	87.2
10000	93.7	93.2	92.3	91.2	89.9
20000	98.3	97.6	96.3	95.0	93.5
40000	104.2	103.3	101.2	99.7	97.6
60000	107.3	106.2	104.1	102.2	99.6
80000	109.0	107.7	105.1	102.9	100.0
k_1(sec.$^{-1}$)	3.7×10^{-3}	2.1×10^{-3}	1.3×10^{-3}	8.2×10^{-4}	5.0×10^{-4}
$\log_{10}k_1$	(-2.43)	-2.68	-2.90	-3.09	-3.31
$\frac{1}{T}\times10^3$	2.597	2.646	2.681	2.717	2.755

TABLE VII(g)

The temperature dependence of the rate of decarboxylation of 2-keto-L-galactonic acid in 19% (w/w) hydrochloric acid.

Time (sec.)	% moles CO_2 per mole acid			
	112°C	105°C	100°C	95°C
1000	43.3	25.0	19.0	14.9
2000	69.6	49.8	36.8	28.4
4000	93.1	83.6	68.4	51.0
6000	99.6	95.3	86.5	69.0
8000	102.5	99.3	94.2	75.9
10000	104.2	101.2	97.9	91.1
14000	105.9	102.9	101.0	97.6
20000	107.6	104.8	103.0	101.4
40000	111.2	108.6	107.2	105.6
60000	113.8	111.4	110.1	107.9
80000	116.0	113.6	112.4	110.4
k_1(sec.$^{-1}$)	6.8×10^{-4}	3.6×10^{-4}	2.7×10^{-4}	1.6×10^{-4}
$\log_{10}k_1$	-3.17	-3.44	-3.58	-3.79
$\frac{1}{T}\times10^3$	2.597	2.646	2.681	2.717

TABLE VII(h)

The temperature dependence of the rate of decarboxylation of calcium-2-keto-D-gluconate in 19% (w/w) hydrochloric acid.

Time (sec.)	% moles CO_2 per mole			
	112°C	105°C	100°C	95°C
1000	57.1	28.9	19.0	14.0
2000	75.0	52.1	37.4	28.6
4000	85.0	75.5	65.2	51.3
6000	89.5	83.1	78.1	66.9
10000	94.1	89.6	87.6	81.1
14000	95.7	93.1	91.0	87.7
20000	97.4	95.2	93.6	92.0
40000	100.1	99.9	98.3	96.7
60000	104.1	102.7	101.6	99.1
80000	106.1	104.5	103.3	100.8
$k_1(sec.^{-1})$	8.1×10^{4}	4.2×10^{-4}	2.9×10^{-4}	1.9×10^{-4}
$\log_{10} k_1$	-3.09	-3.38	-3.54	-3.73
$\frac{1}{T} \times 10^{3}$	2.597	2.646	2.681	2.717

TABLE VII(i)

The temperature dependence of the rate of decarboxylation of ascorbic acid in 19% (w/w) hydrochloric acid.

Time (sec.)	% moles CO_2 per mole acid				
	112°C	105°C	100°C	95°C	90°C
1000	66.0	36.3	23.0	15.6	9.9
2000	84.1	59.5	41.1	29.0	19.4
4000	94.6	82.5	66.6	50.5	37.6
6000	97.8	92.5	81.3	66.6	53.8
10000	100.4	98.0	94.5	87.7	75.5
14000	102.3	100.6	98.2	95.3	86.4
20000	104.1	103.1	101.5	99.6	94.3
40000	107.8	107.4	106.2	105.0	102.0
60000	110.1	109.4	108.0	106.3	104.3
80000	111.5	110.3	108.9	107.5	105.4
k_1(sec.$^{-1}$)	1.0×10^{-3}	4.6×10^{-4}	2.8×10^{-4}	1.7×10^{-4}	1.2×10^{-4}
$\log_{10}k_1$	-2.99	-3.34	-3.55	-3.77	-3.93
$\frac{1}{T}\times10^3$	2.597	2.646	2.681	2.717	2.755

Fig.4 is a plot of $\log_{10} k_1$ against $\frac{1}{T}$, the slope of which gives, by calculation, the values of activation energy and A factor. The values obtained are summarised in Table VII(k).

The values of $\Delta S^{\neq}$, the entropy of activation, were found by substituting the values of A in the Eyring equation (141) for a unimolecular reaction

$$k_1 = e\frac{kT}{h} \cdot e^{-E/RT} \cdot e^{\Delta S^{\neq}/R} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (13)$$

where k_1 = the rate constant for the reaction

e = base of natural logarithms

k = Boltzmann's constant

T = absolute temperature

h = Planck's constant

E = Arrhenius' activation energy.

So that from (11) and (13)

$$A = e.\frac{kT}{h} \cdot e^{\Delta S^{\neq}/R} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (14)$$

$$\text{or } \Delta S^{\neq} = 2.303R \cdot \log_{10}\frac{Ah}{ekT} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (15)$$

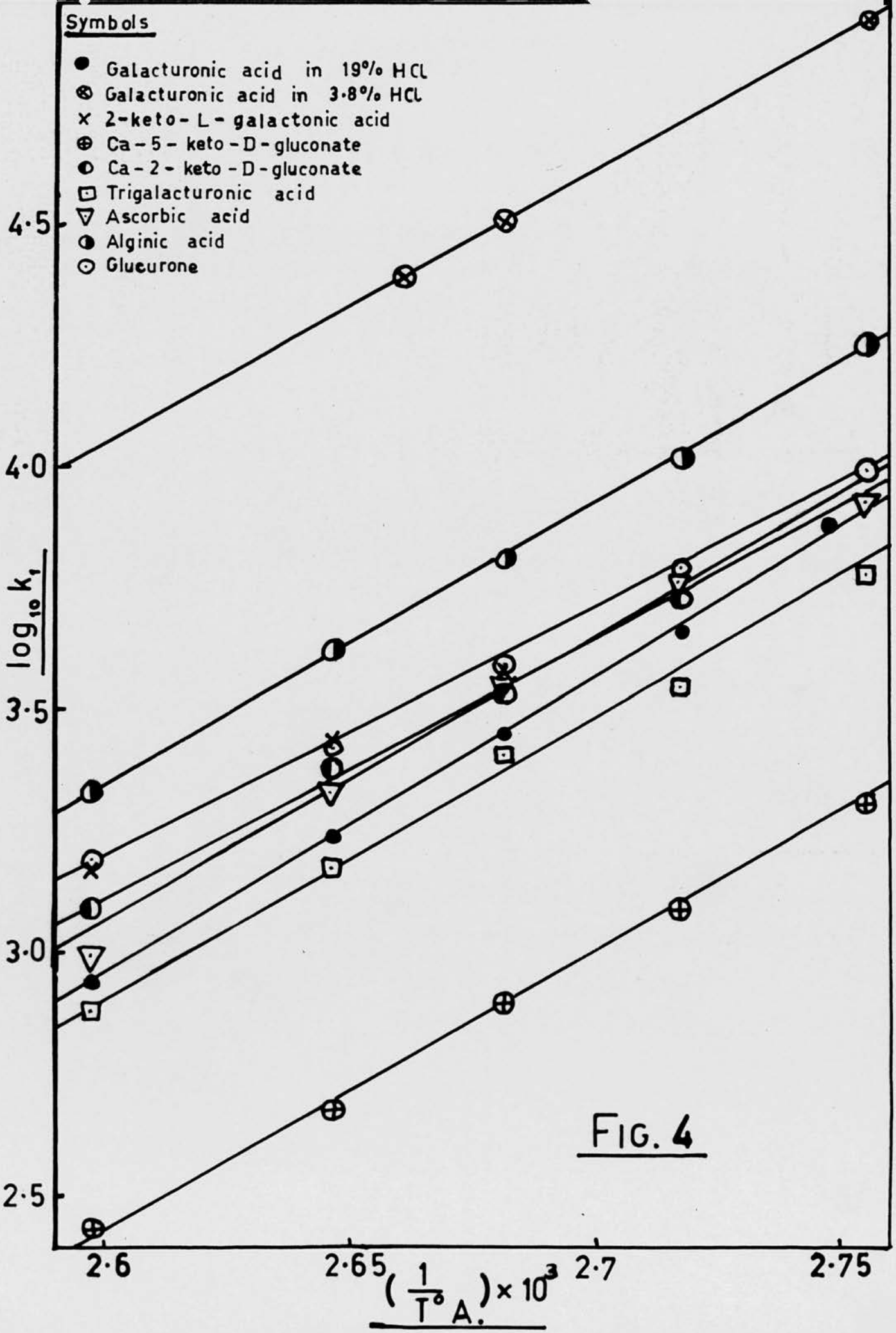

Symbols
Galacturonic acid in 19% HCl
Galacturonic acid in 3·8% HCl
2-keto-L-galactonic acid
Ca-5-keto-D-gluconate
Ca-2-keto-D-gluconate
Trigalacturonic acid
Ascorbic acid
Alginic acid
Glucurone
4·5
4·0
3·5
3·0
2·5
log10 k1
2·6
2·65
2·7
2·75
(1/T° A.) × 10³

FIG. 4

TABLE VII(k)

Arrhenius activation energies and A factors for the decarboxylation of some uronic acids and related compounds in mineral acid.

Substance	Arrhenius activation energy E_A (K cals.)	Arrhenius frequency factor A ($sec.^{-1}$)	Entropy of activation $S^{\neq}$ (cals./deg./mole)
Glucurone in 19% HCl	23.8	2.2×10^{10}	-13.2
Galacturonic acid monohydrate in 19% HCl	27.9	8.1×10^{12}	-1.5
Galacturonic acid monohydrate in 3.8% HCl	25.5	2.7×10^{10}	-12.8
Trigalacturonic acid in 19% HCl	28.0	1.2×10^{13}	-0.76
Alginic acid in 19% HCl	26.5	5.4×10^{11}	-6.9
Ca-5-keto-D-gluconate in 19% HCl	26.4	3.7×10^{11}	-7.6
2-keto-L-galactonic acid in 19% HCl	23.8	2.2×10^{10}	-13.2
Ca-2-keto-D-gluconate in 19% HCl	27.1	2.0×10^{12}	-4.3
Ascorbic acid in 19% HCl	26.1	2.2×10^{12}	-4.1

Fig.4 shows the values of $\log_{10} k$, plotted against the reciprocal of the absolute temperature, the slope of the line giving the activation energy and also the A factor.

It is seen that Ca-5-keto-D-gluconate decarboxylates much faster than any of the other compounds, although its values of activation

energy and of A factor are similar to those of the other uronic acids. The rest of the compounds all give approximately the same rates of reaction. Alginic acid, being a polymer, decarboxylates slower, indicating the stability of the compound. It is possible that the activation energy measured in this case is that of the breaking of the glycosidic links. The activation energy for the hydrolysis of pectin, as found by Weber (73) was 23.8 Kcals. The only other values in the literature are those given by Huber (44) whose results have been summarized previously (p.41). The activation energies for D-galacturonic acid and D-mannuronic acid quoted by him are very much higher than those given above, while his comparable rate constants are very much lower. Application of the Guggenheim method to Huber's results gave similar values to those calculated by him using a simple first order equation. However, only two temperatures were used, and errors due to time-lag in the apparatus could be relatively large. Moreover, liquid scrubbers, which were completely avoided in the present work, were used by Huber, and this could lead to large errors in the rate as measured by the gas collected in a given time; Huber did not decarboxylate any keto acids. Regna and Caldwell (102), however, investigated the formation of ascorbic acid from 2-keto-hydroxy acids and of furfural from ascorbic acids at 60°C and 70°C in 5N.HCl. Rate constants and activation energies were obtained for these reactions and are shown in Table VII(m).

Some of the keto-acids studied by Regna and Caldwell were decarboxylated in the present work, the results being included in Table VII(k). Although the experimental conditions used were different, (112°C as against 70°C, and 19% (w/w) acid (~6N.) as against 5N.), the values found for 2-keto-L-galactonic acid, 2-keto-D-gluconate-Ca-salt and L-ascorbic acid agree very well with those found by Regna and Caldwell, who, in addition, used entirely different methods of assay.

TABLE VII(m)

Kinetics of ascorbic acid formation from 2-keto-hydroxy acids at 59.9°C and 69.9°C in 5N.HCl. (102).

2-keto-acid	k_1 (min.$^{-1}$) at 59.9°C.	k_1 (min.$^{-1}$) at 69.9°C.	E Kcal./mole
L-gulonic	2.53×10^{-3}	8.20×10^{-3}	26.7
D-gluconic	5.66×10^{-4}	1.86×10^{-3}	27.0
D-galactonic	6.54×10^{-4}	2.08×10^{-3}	26.3
D-glucoheptonic	4.78×10^{-4}	1.64×10^{-3}	28.0
D-galactoheptonic	2.07×10^{-4}	7.04×10^{-3}	27.7

TABLE VII(n)

Kinetics of furfural formation from ascorbic acid at 59.9°C and 69.9°C in 5N.HCl. (102)

Acid	k_2(min.$^{-1}$) at 59.9°C	k_2(min.$^{-1}$) at 69.9°C	E Kcal./mole
L-ascorbic acid	4.91×10^{-4}	1.41×10^{-3}	23.9
D-araboascorbic acid	1.62×10^{-3}	3.88×10^{-3}	19.9
D-glucoascorbic acid	9.20×10^{-4}	2.79×10^{-3}	25.2
D-galactoascorbic acid	3.29×10^{-3}	9.43×10^{-3}	23.9

This independent agreement of results implies that the values found for uronic acids are preferable to those quoted by Huber, whose apparatus must be considered to be of poor design for the experiments involved. A further discussion of these results will be given later (see Part VII).

(iv). Investigation of the relationship between acid concentration and rate of decarboxylation.

Although it was well known (65) that the rate of decarboxylation of a uronic acid increased with increasing acid concentration, no investigation had been carried out to determine the exact relationship

between rate and hydrogen ion concentration or acidity. Since some authors recommend the use of 19% (w/w) HCl (92) (93) (94), while others prefer 12% (83) (86) or 20.24% (70), it was of interest to compare the rates of decarboxylation with various strengths of acid. It was considered possible that a relationship might exist between the log of the rate constant, i.e. log k_1, and the Hammett acidity function, H_o, instead of between k_1 and the hydrogen ion concentration, C_{H^+}. If so, some indication of the initial step in the reaction and also of the rate controlling step (103) would be obtained.

The use of the Hammett function can be discussed as follows. Acidity measurements are usually expressed in terms of pH, $-\log C_{H^+}$ (i.e. often written $-\log[H^+]$). In the case of a base B in equilibrium with its conjugate acid BH^+, the concentration of B can be found in the solution by suitable measurements e.g. if B is a coloured base and BH^+ is not coloured.

The equation for this reaction will be

$$BH^+ \rightleftharpoons H^+ + B \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (16)$$

and the equilibrium constant, K_a, is related to the pH by the following equation

$$pH = pK_a + \log \frac{[\text{base}]}{[\text{acid}]} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (17)$$

where $pK_a = -\log K_a$

$$\text{i.e.} \quad pK_a = pH - \log \frac{C_B}{C_{BH^+}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (18)$$

in dilute solution where C_B and C_{BH^+} are the concentration of base and conjugate acid respectively, and

$$K_a = \frac{C_B \cdot C_{H^+}}{C_{BH^+}} \cdot \frac{f_B \cdot f_{H^+}}{f_{BH^+}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (19)$$

where f_B, f_{H^+} and f_{BH^+} are the activity coefficients of B, H^+ and BH^+ respectively. We can then define

$$h_o = K_a \frac{C_{BH^+}}{C_B} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (20)$$

which by substitution in (19) gives

$$h_o = C_{H^+} \cdot \frac{f_B \cdot f_{H^+}}{f_{BH^+}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (21)$$

$$= a_{H^+} \cdot \frac{f_B}{f_{BH^+}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (22)$$

Since B and BH^+ differ by only a proton it could be expected that $\frac{f_B}{f_{BH^+}}$ will be independent of the nature of B. If this is so, h_o will be a property of the solution and will, in fact, define the acidity of the solution as measured by the tendency of a base to take up a proton from that solution. In a similar way to defining pH, H_o is defined by the following

$$H_o = -\log_{10} h_o = pK_a + \log_{10} \frac{C_B}{C_{BH^+}} \quad \ldots\ldots\ldots\ldots \quad (23)$$

Brönsted, (104), by consideration of the reactions of the

cobaltammines, postulated that in an equilibrium

$$A + B \rightleftharpoons C + D \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (24)$$

the rate of the forward reaction v_1 is

$$v_1 = k_1[A][B]\cdot\frac{f_A \cdot f_B}{f_X} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (25)$$

where k_1 = rate constant; [A] and [B] the concentrations of A and B respectively with f_A and f_B their activity coefficients. f_X is the activity coefficient of the critical intermediate complex.

Zucker and Hammett (105) investigating the acid catalysed iodination of acetophenone in solutions of perchloric acid up to 3.6M. found that a plot of log k versus log C_{H^+} was more closely linear with unit slope than the corresponding plot with H_o. On the basis of this and other reactions they put forward the hypothesis that, in general, for unimolecular acid reactions (i.e. A1 reactions) (106), for uncharged reactants, a plot of log k_1 will be linear with H_o and have approximately unit slope. Furthermore, bimolecular acid reactions (i.e. A2 solvolytic reactions) for uncharged reactants, will show linearity between log k_1 and log C_{H^+}, again with approximately unit slope.

A1 reaction (107). This involves a first order rate-determining reaction of the conjugate acid. If S is the substrate

$$\left.\begin{array}{lll} S + H^+ \rightleftharpoons SH^+ & \text{Equilibrium} & \\ SH^+ \rightarrow X^+ & \text{Rate-determining} & \text{A1} \\ X^+ + H_2O \rightarrow \text{products} + H^+ & \text{Fast} & \end{array}\right\}$$

Applying the Brönsted equation (25)

$$\text{the overall rate} = -\frac{dC_S}{dt} = k^1[SH^+]\,\frac{f_{SH^+}}{f_X} \quad \ldots\ldots\ldots\ldots\ldots\ldots (26)$$

Now if K_{SH^+} is the equilibrium constant for the dissociation

$$SH^+ \rightleftharpoons S + H^+$$

$$K_{SH^+} = \frac{c_S \cdot c_{H^+}}{c_{SH^+}} \cdot \frac{f_S \cdot f_{H^+}}{f_{SH^+}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (27)$$

Substituting in (26) from (27) for C_{SH^+}

$$\text{Rate} = -\frac{dC_S}{dt} = \frac{k^1}{K_{SH^+}} \cdot \frac{f_S \cdot f_{H^+} \cdot C_S \cdot C_{H^+} \cdot f_{SH^+}}{f_{SH^+} \cdot f_X} \quad \ldots\ldots\ldots (28)$$

$$\therefore \quad -\frac{dC_S}{dt} = \frac{k^1}{K_{SH^+}} \cdot C_S \cdot \frac{a_{H^+} \cdot f_S}{f_X} \quad \ldots\ldots\ldots\ldots\ldots (29)$$

Now if k_1 is the first-order rate coefficient

$$k_1 \equiv -\frac{1}{C_S \text{ total}} \cdot \frac{dC_S \text{ total}}{dt} \quad \ldots\ldots\ldots\ldots\ldots (30)$$

which by substitution from (29) for $-\frac{dC_S}{dt}$ becomes,

since $C_S \simeq C_S^{\text{total}}$

$$k_1 = \frac{k^1}{K_{SH^+}} \cdot \frac{a_{H^+} \cdot f_S}{f_X} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (31)$$

Now $h_o = \frac{a_{H^+} \cdot f_B}{f_{BH^+}}$ ..... (by definition) ..... (22)

$$\therefore \quad k_1 = \frac{k^1}{K_{SH^+}} \cdot h_o \cdot \frac{f_{BH^+} \cdot f_S}{f_B \cdot f_X} \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (32)$$

$$\text{or } \log k_1 = H_o + \log \frac{f_{BH^+} \cdot f_S}{f_B \cdot f_X} + \text{const.} \qquad \ldots\ldots\ldots\ldots \quad (33)$$

So that $\log k_1 \propto H_o$ for an A1 reaction, provided $\frac{f_S}{f_X} = \frac{f_B}{f_{BH^+}}$. This assumption is reasonable if f_X varies in a similar way to f_{SH^+}. This dependence of $\log k_1$ on H_o shows that <u>a water molecule is not involved in the formation of the transition state</u>.

There is, however, one other possibility, since an S_E2 reaction would also show the same dependence of $\log k_1$ on H_o as for an A1 reaction. In an S_E2 reaction i.e. an acid catalysed bimolecular electrophilic substitution reaction

$$\left.\begin{array}{ll} S + H^+ \longrightarrow SH^+ & \text{Rate-determining} \\ SH^+ + Y \longrightarrow \text{products} + H^+ & \text{Fast} \end{array}\right\} A - S_E2$$

$$\text{Rate} = -\frac{dC_S}{dt} = k_2 C_S \cdot C_{H^+} \cdot \frac{f_S \cdot f_{H^+}}{f_X} \qquad \ldots\ldots\ldots\ldots\ldots\ldots \quad (34)$$

where k_2 is the rate coefficient for proton transfer. Since, in large excess of acid, the reaction will be kinetically of first-order, the first-order rate constant is then

$$k_1 = -\frac{1}{C_S} \cdot \frac{dC_S}{dt} = k_2 a_{H^+} \cdot \frac{f_S}{f_X} = k_2 \cdot h_o \cdot \frac{f_{BH^+} \cdot f_S}{f_B \cdot f_X} \qquad \ldots\ldots\ldots\ldots \quad (35)$$

$$\text{or } \log k_1 = -H_o + \log \frac{f_{BH^+} \cdot f_S}{f_B \cdot f_X} + \text{const.} \quad \ldots\ldots\ldots\ldots (36)$$

So $\log k_1 \propto H_o$ provided the second term is constant. Therefore the same result is reached for an S_E2 reaction as for an A1 reaction (see p. 78). The deduction that water molecules do not participate in complex formation cannot, however, be made in the S_E2 case. It is, therefore, of importance to distinguish which of the two possibilities, i.e. S_E2 or A1 is in fact operative: this is not easy, but evidence from such studies as inversion of configuration and isotope exchange should be helpful (103). This however is beyond the scope of the present work; all that could be done here at this stage is to discover whether the reaction follows an A1/S_E2 or A2 course.

It was decided, therefore, to investigate the exact relationship between the rate constants for the decarboxylation of various uronic acids and the mineral acid concentration. The results are given below. The values of H_o quoted in Tables VIII(a) - (g) were provided in a review by Paul and Long (103) and they hold for a temperature of 25°C. Comparison with the values given by Gel'bstein et al. (108) shows that the values of H_o decrease approximately linearly as the temperature rises; the values used will therefore be larger than the correct values, and this will lead to a slope of less than unity being given for a reaction in which $\log k_1$ depends on H_o.

TABLE VIII(a)

The decarboxylation of D-glucurone in various concentrations of hydrochloric acid.

Time (sec.)	% moles CO_2 per mole uronic acid						
	20.24% HCl	19.0% HCl	15.2% HCl	12.0% HCl	11.4% HCl	7.6% HCl	3.8% HCl
1000	60.0	52.5	26.0	14.8	12.5	4.4	-
2000	77.9	74.9	44.9	28.0	24.4	8.9	3.6
3000	87.2	84.1	59.6	40.0	35.4	13.5	-
4000	92.4	90.0	71.2	42.3	44.4	17.9	7.7
6000	95.9	94.8	84.4	64.6	59.9	27.2	11.5
8000	97.2	96.4	90.0	75.0	71.8	36.2	15.0
9000	97.9	97.0	91.4	79.5	76.6	40.4	16.9
12000	98.5	98.0	94.1	88.3	86.6	50.6	22.5
15000	99.0	98.8	96.0	93.4	91.6	58.7	27.6
20000	100.3	99.7	98.1	96.9	96.3	-	37.0
30000	102.6	101.8	101.0	99.3	98.4	85.9	54.0
40000	104.2	103.8	102.3	100.7	-	-	67.3
50000	105.8	105.5	103.1	102.2	-	-	76.6
60000	107.0	106.5	103.9	102.7	102.0	98.5	83.1
70000	107.6	107.2	104.2	103.1	-	-	87.3
80000	108.0	107.6	104.4	103.5	102.8	100.9	90.3
$k_1(sec.^{-1})x10^4$	8.9	6.5	3.3	1.7	1.5	0.55	0.16
$\log k_1$	-3.05	-3.19	-3.48	-3.77	-3.83	-4.26	-4.70
$-H_o$	2.16	2.00	1.57	1.22	1.15	0.74	0.22
C_{H^+}	2.46	2.20	1.55	1.18	1.13	0.88	0.75
$\log C_{H^+}$	0.391	0.342	0.190	0.072	0.053	-0.056	-0.125

TABLE VIII(b)

The decarboxylation of D-galacturonic acid monohydrate in various concentrations of hydrochloric acid.

Time (sec.)	% moles CO_2 per mole uronic acid						
	20.24% HCl	19.0% HCl	15.2% HCl	12.0% HCl	11.4% HCl	7.6% HCl	3.8% HCl
1000	76.1	67.3	33.5	20.8	16.0	10.0	-
2000	89.8	86.0	58.5	27.2	30.5	20.0	7.9
3000	92.1	91.2	72.8	49.6	43.0	28.9	-
4000	93.7	93.0	79.9	59.5	54.4	37.4	15.0
6000	95.7	94.9	86.5	73.2	70.3	52.3	21.0
8000	96.4	95.5	90.0	83.2	81.0	63.0	27.0
9000	96.8	96.0	91.7	86.0	84.6	67.7	30.0
12000	97.7	97.4	94.2	90.8	90.2	78.7	38.5
15000	98.5	98.2	95.8	91.9	91.5	86.2	46.2
20000	100.0	99.6	97.5	96.8	96.0	92.4	57.4
30000	101.9	101.4	99.6	99.2	98.9	98.0	72.3
40000	103.9	103.0	101.2	101.1	101.0	100.4	82.2
50000	104.9	104.2	102.7	102.5	101.9	101.9	89.1
60000	106.7	105.8	104.0	103.3	102.9	103.1	94.0
70000	107.9	106.8	105.2	104.6	104.5	104.1	97.1
80000	109.0	108.0	106.2	105.6	105.4	105.0	98.2
$k_1 \times 10^4$ (sec.$^{-1}$)	16.6	11.5	4.9	2.5	2.0	1.3	0.40
$\log_{10} k_1$	-2.78	-2.94	-3.31	-3.60	-3.70	-3.89	-4.39
$-H_o$	2.16	2.00	1.57	1.22	1.15	0.74	0.22

TABLE VIII(c)

The decarboxylation of trigalacturonic acid in various concentrations of hydrochloric acid.

Time (sec.)	% moles CO_2 per mole uronic acid monomer					
	20.24% HCl	19.0% HCl	15.2% HCl	11.4% HCl	7.6% HCl	3.8% HCl
1000	76.1	73.0	54.7	25.0	11.7	-
2000	86.4	85.1	77.7	43.4	21.9	9.9
4000	90.8	90.1	88.3	66.9	41.2	18.1
6000	93.0	92.5	90.2	78.6	55.8	27.1
10000	95.5	95.1	93.6	89.7	75.6	42.5
14000	97.2	96.8	95.4	93.3	85.2	55.1
20000	99.4	99.2	97.5	96.3	92.4	69.6
40000	105.3	104.9	103.2	101.8	98.8	93.1
60000	108.9	108.1	106.3	104.4	104.1	101.3
80000	110.9	110.0	108.1	106.0	105.8	104.9
$k_1 x 10^4 (sec.^{-1})$	14.7	13.3	8.9	3.1	1.5	0.51
$\log_{10} k_1$	-2.80	-2.88	-3.05	-3.50	-3.84	-4.29
$-H_o$	2.16	2.00	1.57	1.15	0.74	0.22

TABLE VIII (d)

The decarboxylation of alginic acid in various concentrations of hydrochloric acid.

Time (sec.)	% moles CO_2 per mole uronic acid					
	20.24% HCl	19.0% HCl	15.2% HCl	11.4% HCl	7.6% HCl	3.8% HCl
1000	49.1	33.9	19.8	8.4	5.3	-
2000	72.8	59.0	34.9	16.5	10.7	4.3
4000	84.9	82.7	57.7	32.2	19.9	8.8
6000	94.4	91.2	71.3	45.6	28.4	12.9
9000	99.0	97.1	81.0	62.7	38.1	18.9
12000	100.9	99.5	87.0	74.2	47.7	23.4
15000	101.8	101.1	90.6	81.1	54.7	27.9
20000	104.2	103.0	93.5	88.0	64.2	34.9
40000	109.5	107.7	100.8	97.8	89.1	57.4
60000	113.0	110.0	104.3	101.1	98.5	73.6
80000	115.2	111.0	106.0	103.2	101.2	83.5
$k_1 x 10^4 (sec.^{-1})$	6.3	4.6	2.5	1.3	0.52	0.22
$\log_{10} k_1$	-3.20	-3.33	-3.61	-3.95	-4.28	-4.65
$-H_o$	2.16	2.00	1.57	1.15	0.74	0.22

TABLE VIII(e)

The decarboxylation of calcium-5-keto-D-gluconate in various concentrations of hydrochloric acid.

Time (sec.)	% moles CO_2 per mole gluconate				
	19.0% HCl	15.2% HCl	11.4% HCl	7.6% HCl	3.8% HCl
500	75.0	71.9	55.4	34.5	-
1000	83.5	82.8	76.5	54.9	21.0
2000	87.2	86.6	86.2	74.3	38.3
3000	88.7	88.2	87.8	82.3	53.0
4000	89.4	89.1	88.7	85.3	65.1
6000	90.9	90.0	91.5	89.0	77.2
10000	93.7	93.6	93.3	92.2	88.4
20000	98.3	98.3	98.0	97.4	96.1
40000	104.2	103.4	101.8	101.8	100.9
60000	107.3	106.8	104.8	104.8	103.4
80000	109.0	108.3	107.7	106.3	105.0
$k_1 \times 10^4 (sec.^{-1})$	36.6	36.3	20.5	9.4	2.8
$\log_{10} k_1$	-2.43	-2.44	-2.68	-3.03	-3.55
$-H_o$	2.00	1.57	1.15	0.74	0.22

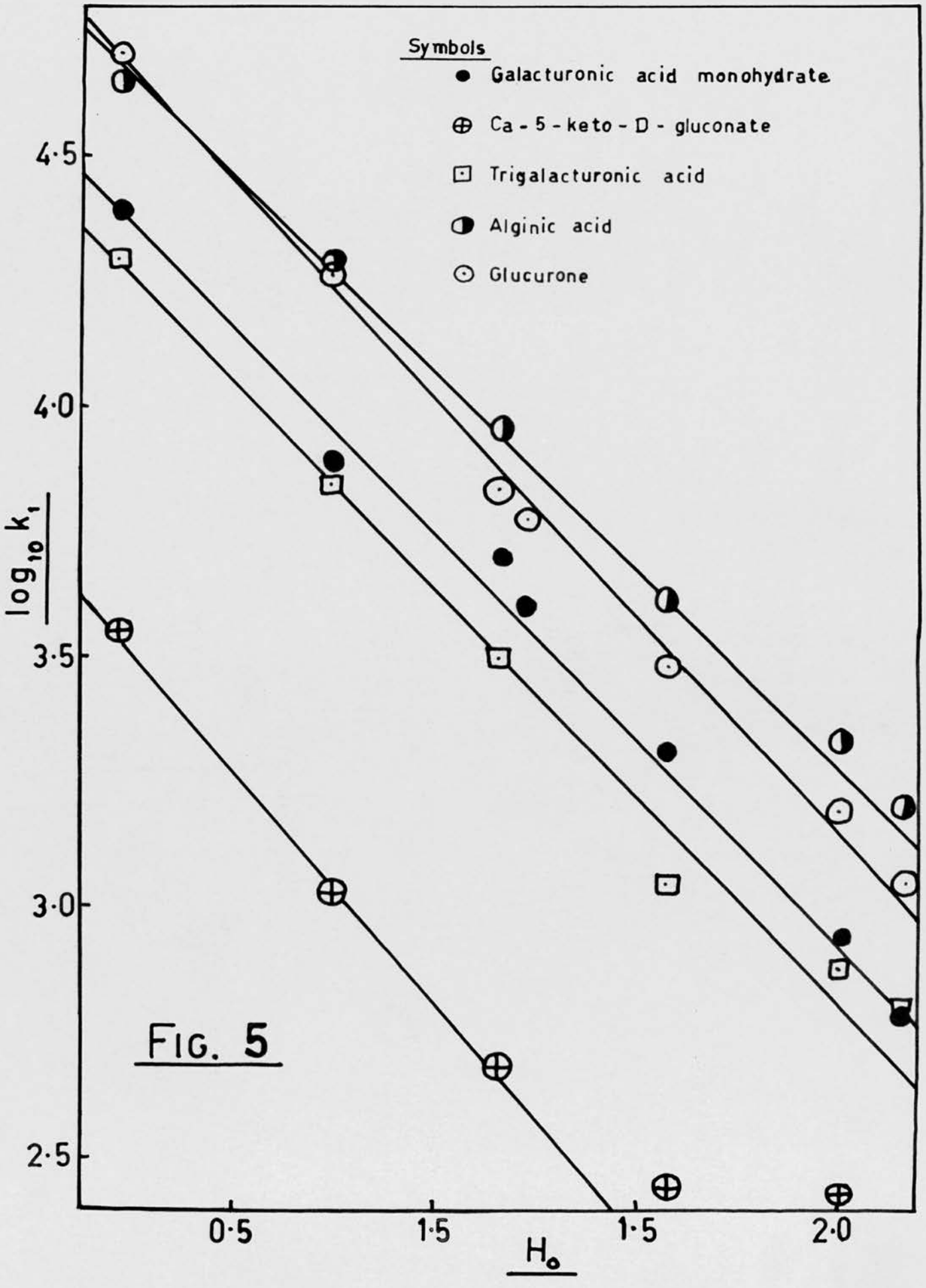

Symbols
Galacturonic acid monohydrate
Ca - 5 - keto - D - gluconate
Trigalacturonic acid
Alginic acid
Glucurone
4·5
4·0
3·5
3·0
2·5
log10 k1
0·5
1·5
1·5
2·0
H0

FIG. 5

TABLE VIII(f)

The relationship between log k_1 and acidity function, H_o.

Substance	Slope of line
D-glucurone	0.93
Ca-5-keto-D-gluconate	0.93
D-galacturonic acid monohydrate	0.85
Trigalacturonic acid	0.85
Alginic acid	0.80

As can be seen from Fig.5, the plots of log k_1 versus H_o give good straight lines and, as expected, the values for the slopes are less than unity.

As indicated in the introduction (Table I), decarboxylation of uronic acids can be carried out in other mineral acid solutions. Table VIII(g) shows the results obtained for sulphuric acid on galacturonic acid monohydrate.

TABLE VIII(g)

Decarboxylation of galacturonic acid monohydrate in various concentrations of sulphuric acid.

Time (sec.)	% moles CO_2 per mole uronic acid					
	50% H_2SO_4	40% H_2SO_4	30% H_2SO_4	20% H_2SO_4	10% H_2SO_4	5% H_2SO_4
1000	90.2	72.2	47.6	19.5	-	-
2000	95.6	91.0	70.9	33.0	12.0	6.5
4000	96.9	96.2	89.9	52.6	22.3	14.2
6000	98.9	98.3	95.4	67.6	31.2	20.0
8000	101.0	99.9	97.6	77.8	39.0	25.3
10000	102.4	101.3	98.4	86.3	46.0	30.4
12000	103.4	102.1	99.2	91.5	52.3	34.9
20000	106.9	104.8	101.5	98.0	70.9	53.1
40000	112.9	109.5	105.4	104.0	95.9	82.3
60000	116.6	112.6	108.7	107.1	104.0	97.3
80000	118.8	114.6	111.2	108.8	106.5	104.0
$\log k_1$	-2.61	-2.79	-3.20	-3.75	-4.27	-4.54
$-H_o$	3.38	2.41	1.72	1.01	0.31	$\sim$0.02

The values for 5-30% H_2SO_4 fall on a straight line when $\log k_1$ is plotted against H_o.

It is also of interest that the values for $\log k_1$ at a given value of H_o are approximately equal for sulphuric and hydrochloric acids. Decarboxylation is also found to be complete in 2½ hrs. when 30% H_2SO_4 is used. This indicates that the same mechanism is operative for both acids.

There is, however, a slight "tailing off" of the straight lines at higher values of H_o suggesting that a maximum value of k was being reached. This type of phenomenon has been found by Schubert (109) during investigation of the rate of decarboxylation of mesitoic acid, the maximum in that case being given when approx. 80% sulphuric acid was used. However, in cases of reactions with previously verified mechanisms, the presence of an A-1 mechanism is indicated by dependence of log k_1 on H_o (103).

Whilst the tendency to non-linearity observed may be due to experimental error, nevertheless it may indicate either the formation of a less reactive species or the participation of a reaction which is independent of the acidity. Such a phenomenon is more clearly seen when H_3PO_4 is used as decarboxylating agent. A few runs were carried out using this acid; although it is possible to deduce an initial relationship of log k_1 to H_o, the rate goes through a maximum, and it was found, for instance, that the rate of decarboxylation in 80% H_3PO_4 was slower than in 40%.

To clarify the conflicting evidence regarding the possibility of the reaction mechanism changing at a certain acid concentration, a further investigation of the relationship between water content of an acid solution, at a given value of H_o, and rate of decarboxylation should be carried out. This could possibly be done in mixed solvents; unfortunately, insufficient data in the literature at the moment obstructs this line of work. An attempt to decarboxylate D-galacturonic acid monohydrate was made using a dioxan-water mixture containing HCl. At the temperature required to give a measurable rate of decarboxylation, the dioxan also

decomposed, so giving anomalous results.

Long and co-workers have used other kinetic tests for the determination of the molecularity of acid catalysed reactions. They applied (110) Taft's earlier suggestion (142) that for acid catalysed reactions of the same type of compound the entropy of activation, $\Delta S^{\neq}$, for an A-1 reaction will be about 25 entropy units (e.u.) greater than for an A-2 reaction. This corresponds roughly to the entropy of the bound water molecule in the bimolecular reaction. These workers also found that the entropy of activation of an A-1 reaction is often almost zero. The values for $\Delta S^{\neq}$ for several A-1 and A-2 acid catalysed hydrolysis reactions in aqueous solution are given below.

Substrate	Mechanism	$\Delta S^{\neq}$ (e.u.)
Ethyl orthoformate	A-1	+ 5.8
Sucrose	A-1	+ 7.9
Ethylal	A-1	+ 7.3
t-butyl mesitoate	A-1	+ 9
Methyl acetate	A-2	- 21.3
Ethyl acetate	A-2	- 23.0
y-valerolactone	A-2	- 24.6
y-butyrolactone	A-2	- 20.9

A comparison with Table VII(k) shows that the values for the entropies of activation of the uronic acid decarboxylation reaction are all negative, indicating either that the activated complex is more stable than the initial structure or that not all the activated molecules decompose. The latter would tend to suggest a pre-decomposition equilibrium as indicated in the proposed prototype A-1 mechanism (p. 76).

The fact that the negative values of $\Delta S^{\neq}$ (in Table VII(k)) are also relatively small is further evidence for the existence of an A-1 type reaction mechanism. Although the values for $\Delta S^{\neq}$ for glucurone, 2-keto-L-galactonic acid and galacturonic acid monohydrate in 3.8% HCl are greater than those obtained for the other acids examined, they are much smaller than the values required for an A-2 mechanism.

A further test, which has been applied to the acid catalysed hydrolysis of trimethylene oxide, is the value of $\frac{k_D}{k_H}$, i.e. the ratio of the rate constants in light and heavy water (111). In the investigated reactions, the value was usually less than 1.5 for bimolecular and approximately 2 for unimolecular reactions; it was suggested that this difference is due to the differing solvolytic power of H_2O and D_2O. Further investigations using both the entropy of activation and D_2O techniques are therefore required on materials more closely related to uronic acids. Unfortunately, the high cost of DCl (approx. £40 per 5 litres) would make this experiment excessively expensive to carry out at this stage.

Section (v). The decarboxylation of uronic acids in pure water.

The extraction of a polysaccharide from its plant or animal source is always the first step in the elucidation of its structure. Although extraction is often carried out in the cold, the use of boiling water or even of a boiling salt solution may be necessary. It is often the case, however, that investigators neglect the importance of ensuring that the extraction procedure used employs the mildest possible conditions consistent with extraction in reasonable yield of the polysaccharide material required. Fractionation, sub-fractionation, degradation and chemical modification are all possible consequences of carelessly designed extraction schemes.

It was considered useful, therefore, to investigate whether decarboxylation of various uronic acids did in fact occur in pure water, such as is provided by a laboratory deioniser. Decarboxylation was found to take place, and the rate constants were calculated as before assuming first order kinetics. Satisfactory straight line plots were obtained. The use of boiling 70% ethanol in water also gave rise to some decarboxylation. A hot (90°C), 0.5% oxalate solution, made up of 0.25% oxalic acid with 0.25% ammonium oxalate, is the standard medium for the extraction of acidic polysaccharides from the natural material. This was also found to decarboxylate the uronic acids (Table IX(c)).

TABLE IX(a)

The decarboxylation of uronic acids and related compounds in pure water.

Time (sec.)	Glucurone	Galacturonic Acid Monohydrate	Tri-Galacturonic Acid	Alginic Acid	5-keto-D-gluconate	Ascorbic Acid
	% moles CO_2 per mole acid					
10000	5.6	7.8	12.8	3.7	35.0	7.7
20000	11.1	15.1	24.2	7.3	50.9	15.2
30000	16.6	21.9	33.6	11.0	60.2	22.1
40000	21.8	28.3	41.4	14.6	66.4	28.1
60000	31.5	39.8	54.2	21.7	73.3	39.2
80000	40.4	50.0	64.1	28.5	78.3	49.4
100000	48.7	59.2	72.2	35.1	87.4	58.6
150000	66.3	78.2	87.2	50.7	90.2	77.8
200000	79.4	91.4	96.0	65.6	96.4	93.0
250000	88.4	97.8	99.4	79.4	98.3	98.9
$k(sec.^{-1}) \times 10^6$	5.18	6.56	12.55	1.42	57.6	6.28

TABLE IX(b)

The decarboxylation of various uronic acids in 70% ethanol-water solution. (Results as % moles CO_2 per mole)

Time (hrs.)	10	20	30	50	100	150
Galacturonic acid monohydrate	3.9	6.8	9.6	15.2	26.8	38.0
Glucurone	3.4	6.3	9.1	14.6	25.5	35.4
Alginic acid	2.6	4.5	6.6	11.9	22.1	32.2

TABLE IX(c)

The decarboxylation of various uronic acids in a solution of 0.25% oxalic acid + 0.25% ammonium oxalate at 90°C. (Results as % moles CO_2 per mole)

Time (hrs.)	5	10	20	40	60	100
Galacturonic acid monohydrate	5.8	10.1	19.4	36.1	48.8	67.1
Glucurone	5.0	8.4	15.9	29.2	40.2	60.1
Alginic acid	4.6	7.5	14.3	26.6	36.2	53.2

It is therefore seen that the above substances decarboxylate to quite a large extent when boiled in water or in oxalate solution, and also in ethanol-water solution to a much smaller extent. Structural studies on polysaccharides containing uronic acids should therefore take this into account.

Section (vi). The decarboxylation of uronic acids labelled with C-14.

The use of potassium-D-glucuronate-6-C-14 affords a means of obtaining the absolute rate of removal of the carboxyl group without interference from carbon dioxide produced by side reactions. Apart from this, the use of electronic equipment to give the total count of active CO_2 liberated gives the experiment

greater accuracy than could be attained by the usual quantitative chemical methods. Both the rate of decarboxylation and the time required for total liberation of CO_2 from C_6 can be found by taking counts at time intervals.

APPARATUS.

The CO_2 evolved from labelled compounds was passed, along with the nitrogen scrubbing gas, through a scintillation cell, (manufactured by Nuclear Enterprises (G.B.) Ltd.), designed to permit high efficiency counting of C-14 labelled vapours mounted in a photomultiplier. The photomultiplier was attached to a Type 1014A probe unit whose pulse output was counted on a Type 1009B scalor fitted with a three decade electronic register. In preliminary experiments it was found that CO_2 was absorbed on the surface of the scintillation cell giving a large residual count even when no active CO_2 was being liberated. The scintillation cell was, therefore, replaced by a flow-cell of special design (143) in which the mica end-window (1.9 mg./cm.^{2} window density) of a Geiger tube acted as one wall of the cell. The voltage for the Geiger tube was derived from a stabilised power supply Type 1007 with a suitable potentiometer unit. The counting system was set at zero at the start of each run and readings were taken at successive minute intervals. Since the Geiger tube, while not so sensitive in terms of counts as the photomultiplier, showed much better response to changes in the C-14 concentration in the flow-gas, it was used in all subsequent experiments with C-14 labelled compounds. The labelled compounds were obtained from The Radiochemical Centre, Amersham, England.

(a) The use of $BaCO_3$-C14 as a source of labelled CO_2.

One possible error in a uronic acid determination is due to absorption of CO_2 in any water adhering to the condenser surfaces even though the partial pressure of the CO_2 dissolved would be very low in the large excess of scrubbing nitrogen. (Total yield of CO_2 from 30 mg. uronic acid is approx. 4 c.c. at N.T.P.). Since the liberation of CO_2 from labelled barium carbonate by acid is virtually instantaneous, a good measure of the retention of CO_2 by the apparatus was given by the time required to sweep over all the labelled CO_2 evolved. The apparatus had been designed (101) with the intention of minimising the "dead space". The experimental conditions used in this study for uronic acid determinations were reproduced in all respects other than that more dilute acid was used. The reaction flask of Fig.1 was replaced by one having a B10 socket fused into the wall as shown in Fig.6. A small dropping funnel which contained the acid was fitted into the B10 socket.

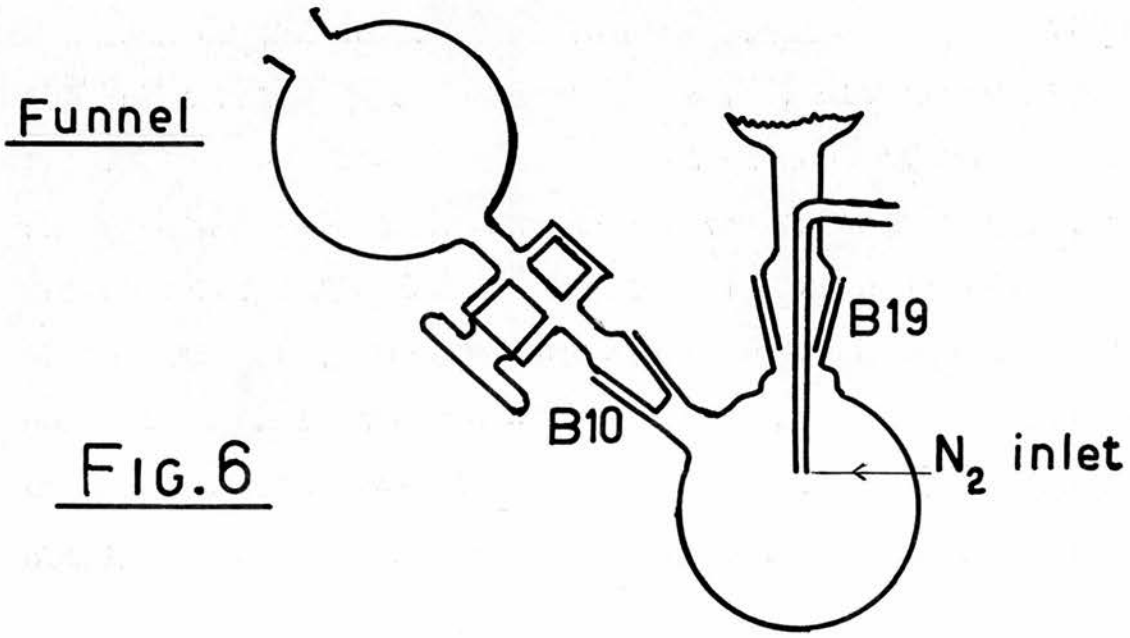


FIG.6

C-14-labelled barium carbonate was weighed out in a small bucket and placed in the reaction flask. On addition of acid from the dropping funnel the liberated CO_2 was passed first through the counting cell and then into baryta. The heater was switched on and the acid allowed to boil as in a uronic acid determination, counts being taken at each minute. Nitrogen was used as scrubbing-gas. The total count was read from the instrument, the counts per min. were obtained by subtraction, the dead-time correction was obtained from tables and hence the total corrected count was estimated.

TABLE X(a)

The liberation of CO_2 from pure C-14-labelled $BaCO_3$.

Wt. of $BaCO_3$-C14 = 7.34 mg.

Wt. of $BaCO_3$ as estimated from baryta used after 2 hrs. = 7.41 mg.

Background count = 47 counts per min.

Time (sec.)	Total count	Count per min.	Dead-time correction	Corrected count per min.	True count per min.	Total corrected count
0	0	0	0	0	0	0
60	47	47	-	47	0	0
300	3400	3539	85	3624	3577	3871
600	40000	9900	701	10601	10554	42373
1200	196500	21000	4454	25454	25407	223139
1800	331600	8300	487	8787	8740	327341
2400	380000	2700	49	2749	2702	422070
3600	398980	300	1	301	251	440265
5400	-	72	-	72	25	-

It can be seen from Table X(a) that all but a trace of CO_2 was swept over in 60 mins. and that only very slight "tailing" occurred. To find out if the liberation of a larger amount of CO_2 affected the tail, a mixture of labelled and unlabelled $BaCO_3$ was used.

TABLE X(b)

Wt. of $BaCO_3$-C14 = 3.26 mg.

Wt. of $BaCO_3$-A.R. grade = 10.50 mg.

∴ Total weight of $BaCO_3$ = 13.76 mg.

Wt. found by titration of excess baryta after 2 hrs. = 13.94 mg.

Background count = 54 counts per min.

Time (sec.)	Total count	Count per min.	Dead-time correction	Corrected count per min.	True count per min.	Total corrected count
0	0	0	0	0	0	0
60	54	54	-	54	54	0
600	22100	7700	417	8117	8063	22555
900	68400	10400	776	11176	11122	71655
1200	111500	6600	304	6904	6850	117190
1800	141500	1300	11	1311	1257	147402
2400	147180	230	1	231	177	152568
3600	149408	65	-	65	11	153718

The results presented in Table X(b) show that all the CO_2 came over in approximately the same time as in the first experiment i.e. Table X(a). This indicates that, within experimental error, the sample size does not affect the accuracy of the determination.

Since 30 mg. samples of uronic acids, yielding 5-7 mg. CO_2 were usually taken in the decarboxylation experiments carried out, the results shown in Table X(b) are more typical than those in Table X(a). The internal swept volume of the apparatus used is approximately 150 ml., so that, using a nitrogen flow-rate stabilised at 15 ml./min., 10 minutes should elapse for the first traces of CO_2 liberated in the reaction to appear. Table X(b) shows, however, that although the total count after 10 minutes is 22555, the count per minute for the 9th-10th minutes is 8063: thus it is unlikely that any CO_2 reached the scrubber much before the 7th minute. These values, when considered in relation to the total count of 153718 obtained, indicate that the apparatus design permitted satisfactory measurements of the rate of liberation of CO_2, particularly when the following factors are considered:-
(a) The data in Table X(b) refers to CO_2 released from a carbonate: the release of CO_2 from uronic acid materials is not so rapid initially; (b) In taking kinetic measurements, no attempt was made to take readings for time intervals shorter than 900 secs.

(b) The decarboxylation of potassium-D-glucuronate-6-C-14 in 19% HCl.

Table X(c) shows the results obtained for the decarboxylation of a sample of potassium-D-glucuronate-6-C-14 in 19% HCl under the same conditions as employed in an actual analysis. The % moles CO_2 per mole of K-D-glucuronate were calculated from the values obtained by the normal titrimetric method, assuming the liberation of 1 mole of CO_2 from 1 mole of glucuronate.

TABLE X(c)

The decarboxylation of potassium-D-glucuronate-6-C-14 in 19% (w/w) HCl.

Wt. of sample = 7.10 mg.

Background count = 54 counts per min.

Time (sec.)	Total count	Count per min.	Dead-time correction	Corrected count per min.	True count per min.	Total corrected count	Corrected time (sec.)	% moles CO_2 per mole of glucuronate
60	54	54	-	54	0	0		
600	1010	204	-	204	150	455		
900	15800	6700	314	7014	6960	15472	0	
1200	71900	13100	1255	14355	14301	76061	300	
1800	208100	12700	1175	13875	13821	225346	900	45.2
2400	317400	9500	643	10143	10089	342793	1500	
3000	391900	6500	295	6795	6741	420697	2100	
3600	442700	4100	115	1215	1161	470760	1700	
4200	473100	2100	30	2130	2176	501370	3300	
4800	488340	1110	8	1118	1064	516229	3900	
5400	496000	570	2	572	518	523367	4500	
7200	506357	227	-	227	173	532227	6300	
9000	511780	150	-	150	96	536038	8100	92.1
10800	516060	140	-	140	86	538698	9900	
12600	519648	106	-	106	52	540660	11700	
14400	523050	110	-	110	56	542442	13500	

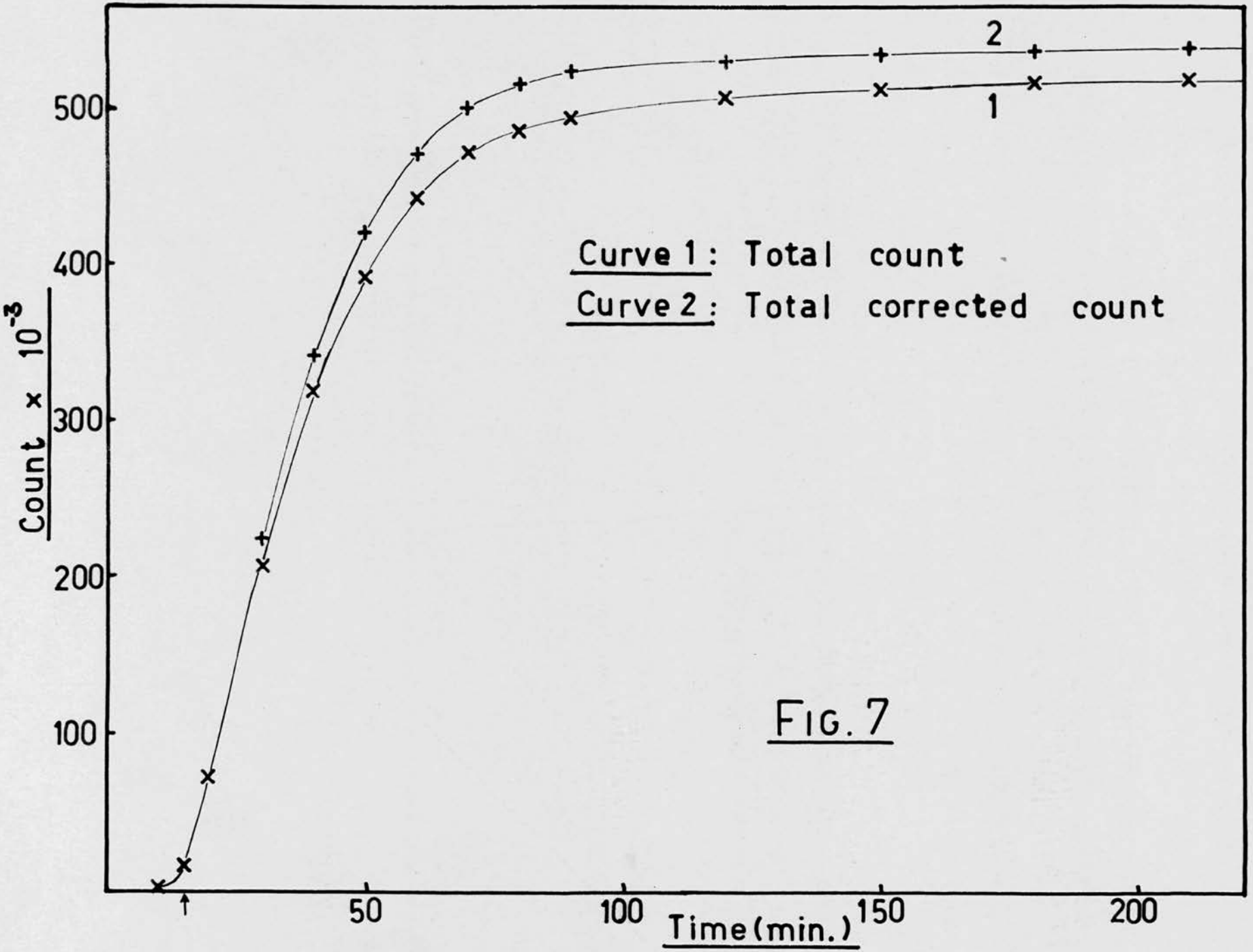

2
1
500
400
300
200
100
Count x 10^{-3}
Curve 1: Total count
Curve 2: Total corrected count
50
100
150
200
Time (min.)

FIG. 7

Fig.7 shows the curve given by the results presented in Table X(c). Experimentally the time when the heater was first switched on was taken as zero, and all the CO_2 liberated from the glucuronate was counted from this time. If the reaction is assumed to be accurately of first-order, then the corrected zero time for the reaction will be obtained by the projection back of the curve to zero counts. This projection gave +900 sec. as the reaction zero time; the corrected time was obtained by subtraction and is shown in Table X(c).

Calculation of the rate constant $\underline{k}_1$ was carried out, using Guggenheim's method, from a straight-line plot of log (counts at $t_1 + \Delta$ - counts at t_1) versus t_1. $\underline{k_1 = 7.4 \times 10^{-4} \text{sec.}^{-1}}$. This is higher than the value, $6.5 \times 10^{-4} \text{sec.}^{-1}$ obtained for glucurone by the titrimetric method (Table VIII(a)).

This is to be expected from the following reasoning.

<u>For titration values</u> (for typical calculation see Table V(b)) $(C_{t_1+\Delta} - C_{t_1}) < 1$, and so the characteristic of the log of this function is $\bar{1}$ or $\bar{2}$ giving a -ve value for the logarithm obtained by subtraction of the +ve mantissa from the -ve characteristic. The extraneous CO_2 will increase the value of $C_{t_1+\Delta}$ more than C_{t_1} and hence increase $(C_{t_1+\Delta} - C_{t_1})$; hence the mantissa will be larger, giving a smaller value of $\log(C_{t_1+\Delta} - C_{t_1})$ on subtraction, than would be the case if the extraneous CO_2 was not involved, as with the C-14 method. Hence it is to be expected that a plot of log $(C_{t_1+\Delta} - C_{t_1})$ versus t_1 will have a smaller slope by the titration method, so giving a lower value of k_1.

After 2½ hrs. (9000 secs.) the true count per minute is 96 out of a total count of 536038 and is decreasing. This indicates that the glucuronate is essentially decarboxylated during this time. The value of % moles CO_2 per mole of uronate compare favourably with the values for glucurone itself (see Table VII(a)), indicating a similar rate of reaction.

(c) Decarboxylation of potassium-D-glucuronate-6-C-14 in 12% (w/w) HCl.

Many authors (see Table I) have recommended the use of 12% HCl as decarboxylating agent with a reaction time of five hours. It was decided to check this reaction time with labelled glucuronate. Table X(d) shows the results obtained.

TABLE X(d)

The decarboxylation of potassium-D-glucuronate-6-C-14 in 12% (w/w) HCl.

Wt. of potassium-D-glucuronate-6-C-14 = 6.05 mg.

Background count = 70 counts per min.

Time (sec.)	Total count	Count per min.	Dead-time correction	Corrected count per min.	True count per min.	Total corrected count	Corrected time (sec.)	% moles CO_2 per mole of glucuronate
60	70	70	-	70	0	0		
900	2650	360	1	361	291	1599	0	
1800	38100	3700	94	3794	3724	36680	600	
2700	89400	3400	79	3479	3409	88128	1500	
3600	138300	3200	79	3274	3204	137183	2400	40.1
5400	223500	2500	42	2542	2472	221931	4200	
7200	294000	2200	33	2233	2163	289088	6000	
9000	351000	1800	22	1822	1752	344723	7800	
10800	395700	1200	10	1210	1140	387774	9600	
12600	416960	490	1	491	421	407041	11400	
14400	439420	690	3	693	623	427498	13200	
16200	457950	550	2	552	482	444000	15000	
18000	472220	430	1	431	361	456309	16800	93.2
19800	482870	290	-	290	220	464885	18600	
21600	490970	250	-	250	180	470887	20400	
23400	494890	150	-	150	80	473653	22200	

Table X(d) shows that the corrected count approximates to the background count after 6½ hours. Calculation by Guggenheim's method shows the rate constant $k_1 = 1.6 \times 10^{-4} sec.^{-1}$. This agrees well with the value of $1.7 \times 10^{-4} sec.^{-1}$ found earlier by titration (Table VIII(a)).

Although the C-14 method shows that decarboxylation is not truly complete in less than 6½ hours, consideration of the total counts shows that, after 5 hours, decarboxylation is approx. 97.5% complete. The value by titration after 5 hours agreed well with the value obtained after 2½ hours in the corresponding experiment using 19% acid (Table X(c)). In determinations, involving titration, on mixed natural product samples it is therefore possible that the spurious CO_2 produced in side-reactions, or from decomposition of non-acidic polysaccharide material, will amount to 2½%, so that the apparent correct result is obtained by the summation of 97½% from true uronic acid decarboxylation and 2½% from the other possible sources.

The influence of this finding on the procedure to be recommended for analysis of natural products will be discussed later.

Section (vii). The analysis of uronic acids.

From the preceding results it is seen that nitrogen at a flow-rate of 15 ml./min. is the best flow-gas, giving the least errors.

The weight of sample taken is unimportant provided the results are calculated as % moles CO_2 per mole uronic acid or as % uronic acid anhydride.

Since there appears to be no advantage in the use of 12% HCl, sulphuric acid, or phosphoric acid, 19% (w/w) HCl is recommended as decarboxylating agent, in which the reaction time, as found by both

titrimetric and labelled CO_2 measurements, is 2½ hours. The titrimetric method used throughout this work appears to be satisfactory for routine analysis.

It is suggested that for polysaccharide materials, in which the constituents have been characterised, a more accurate estimation of uronic acid content can be obtained by subtraction of the % CO_2 liberated from the known non-uronic acid materials, in 19% HCl after 2½ hours, from the value given by the polysaccharide under the same analytical conditions.

A comparison of results obtained by decarboxylation and colorimetric methods is given later (Part VI).

PART II.

The liberation of CO_2 from uronic and non-uronic acid carbohydrate materials.

1) In aqueous solution containing metal ions.

2) In aqueous solution containing antioxidants.

(1) Metals

Introduction

(i) The metal ion catalysed decarboxylation of carboxylic acids.

Heavy metal ions are essential, in traces, for the growth and metabolism of all living cells. The biologically active metals are molybdenum, cobalt, copper, zinc, iron and manganese, and all are essential for plants and animals except cobalt, which is believed not to be essential for plants (122). The metals are usually located in enzyme systems, although chlorophyll, insulin and haemoglobin are examples of non-enzymic locations.

As mentioned in the introduction to Part I, carboxylic acids can be decarboxylated in basic media, although the presence of a metal catalyst is usually required. Polysaccharide materials isolated from plant or animal sources often have significant ash contents; consequently, decarboxylation in strong mineral acid could, if catalysis by metals occurs, give high results for uronic acid content because of overoxidation effects coupled with altered kinetics.

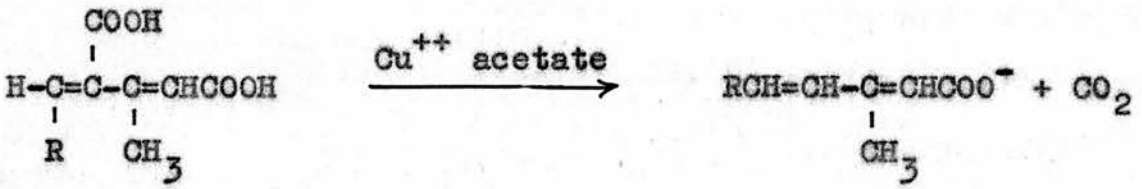
The actual mechanism of metal catalysis is not well understood. One possibility is that the metal acts as an electron acceptor from double bonds in unsaturated systems and also from carbon atoms α- to the carboxyl group, hence facilitating the liberation of CO_2 from that carboxyl group.

Heavy metal ions can form complexes with nucleophilic groups

(e.g. $-NH_2$, $-COOH$, $-\overset{|}{C}-OH$, $-CHO$) so that the molecules acquire an induced positive charge. In carboxylic acids this facilitates the movement of electrons from the carboxyl group, so affecting the tendency for decarboxylation to occur.

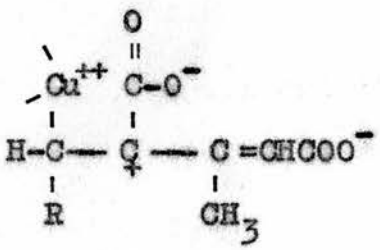
Gilman and Lousinian (112) decarboxylated 2-furan carboxylic acid by heating in a tertiary base in the presence of $CuSO_4$. Copper has a strong tendency to form complexes with electron donor groups.

Unsaturated carboxylic acids can also be decarboxylated; y-arylidene-2-methyl glutaconic acid, LII, is selectively decarboxylated at the y-position by cupric acetate in 2-4-lutidine as solvent (113).



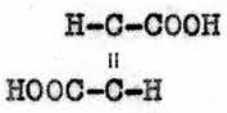
LII LIII

It is suggested that a complex is formed of the type, LIV,

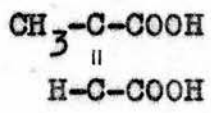


LIV

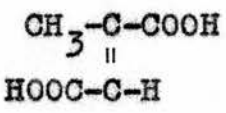
Cu^{++} chelate compounds analogous to LIV have also been postulated for fumaric , LIV(a), citraconic, LIV(b), and mesaconic acids, LIV(c) (114).



LIV(a)



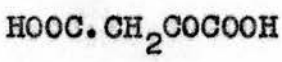
LIV(b)



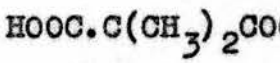
LIV(c)

The study of the metal catalysed decarboxylation of keto acids received considerable impetus when it was found that the biological decarboxylation of β-keto acids was due to metal enzyme systems (115) (116) (117) (118).

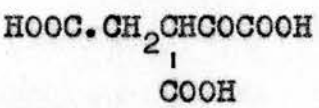
Various authors found that β-keto dicarboxylic acids such as oxaloacetic acid, LV, (119) (118), dimethyl oxaloacetic acid, LVI, (120) (58), oxalosuccinic acid, LVII, (116) and acetone dicarboxylic acid, LVIII, (121) liberated CO_2 on heating in the presence of various metal ion solutions.



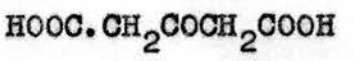
LV



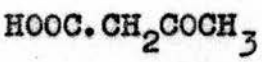
LVI



LVII



LVIII



LIX

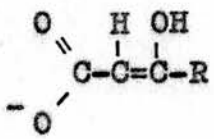
In these dicarboxylic acids only one carboxyl group is removed as CO_2. Aceto-acetic acid, LIX, a β-keto monocarboxylic acid, and α-keto-monocarboxylic acids such as α-keto glutaric acid and pyruvic acid do not decarboxylate under similar reaction conditions (115).

Prue (121) investigating the decarboxylation of acetone dicarboxylic acid in acetate buffer in the presence of metal ion catalysts found the following order of catalytic efficiency:-

$$Cu^{++} > Be^{++} > Al^{+++} > Ni^{++} > Zn^{++} > Co^{++} > La^{+++} > Pb^{++} > Mn^{++} > Cd^{++} > Mg^{++}$$

Speck (118) studied the metal catalysed decarboxylation of oxaloacetic acid, LV, with Zn^{++}, Cu^{++}, Co^{++}, Fe^{++}, La^{+++} salts and found the reaction to be first order and also dependent on the pH of the solution. In very acid (pH 1) and very basic (pH 13) solutions the oxaloacetic acid is stable. This decomposition was also studied by Krebs (115) who compared the decarboxylation of this α-keto acid with that of acetone dicarboxylic acid, i.e. a β-keto acid. Using 3×10^{-3}M. $Al_2(SO_4)_3$ at 20°C and pH 4.4 he found that oxaloacetic acid decarboxylated much faster than the β-carboxylic acid.

A spectroscopic investigation of the catalytic mechanism of the decarboxylation of oxaloacetic- and oxalosuccinic acids was carried out by Kornberg, Ochoa, and Mehler, (123). Investigating the form of the β-keto acid complex they found that it probably contained the acid in the enol form i.e.



They therefore put forward the following reaction scheme:-

(a) β-keto acid ⇌ enol

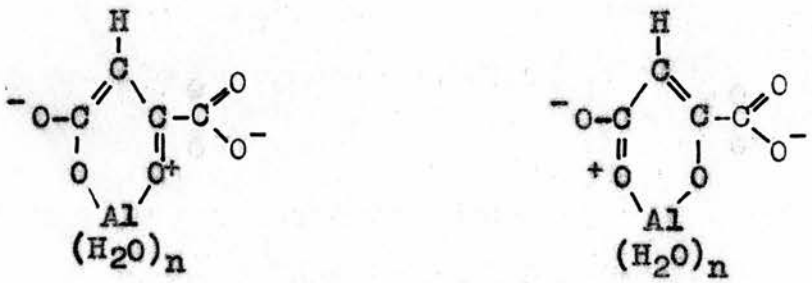
(b) Enol + cation ⇌ complex

(c) Complex ⟶ α-keto acid + CO_2.

In contrast, Martell and Calvin (124) postulated the following cyclic intermediates for the metal-enolate ions of acetoacetic acid, LX, and oxaloacetic acid, LXI, after obtaining evidence that, although both acids formed analogous complexes with Al^{+++}, only oxaloacetic acid underwent decarboxylation.

Acetoacetic acid

LX



Oxaloacetic acid

LXI

Schellenberger (125), studying the effect of metal ions on the rate of decarboxylation of pyruvic acid, found that the order of catalytic effect was

$$Al^{+++} > Cu^{++} > Be^{++} > Cd^{++} > Pb^{++} > Ni^{++} > Zn^{++} > Co^{++} > Mg^{++} > Ca^{++} > Ba^{++}$$

This agrees well with the findings of Pedersen (126) who gave the order of retardation of the decarboxylation of nitro-acetic acid as

$$Al^{+++} > Cu^{++} > Be^{++} > Cd^{++} > Pb^{++} > Ni^{++} > Zn^{++} > Co^{++} > Mg^{++} > Ca^{++} > Ba^{++}$$

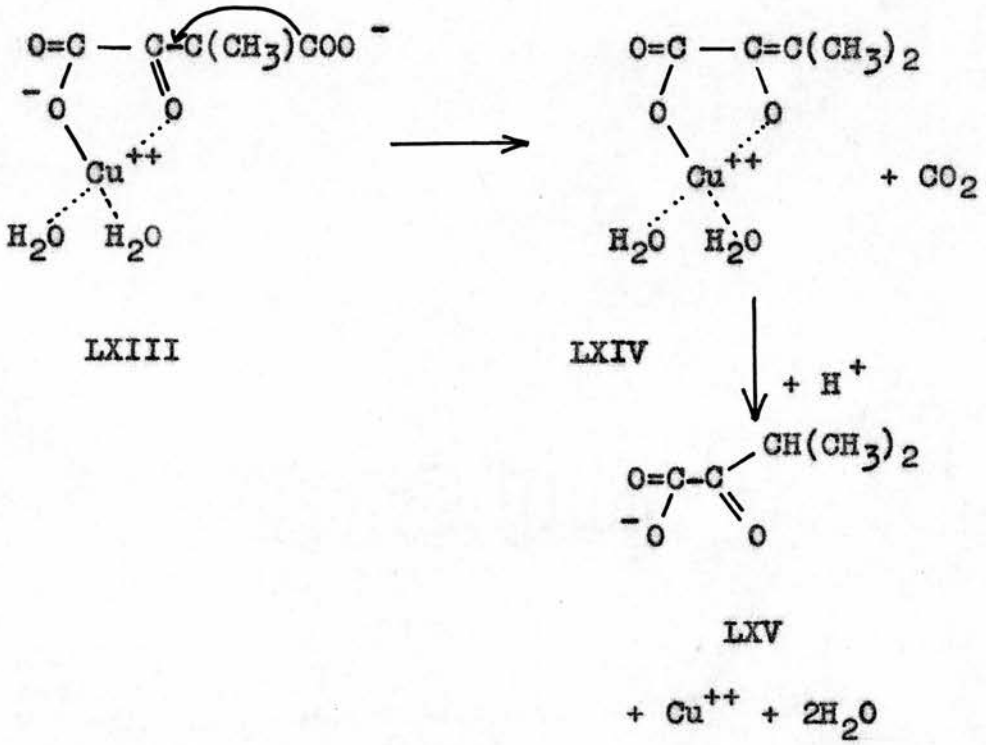
(in this case the metal complex is more stable than the free ion). From these results, Al^{+++} and Cu^{++} form the most stable complexes with both pyruvic and nitro-acetic acids. According to Pedersen (127), formation of a complex between the carboxyl group and the metal ion inhibits the electron shift of the electrons from the carboxyl group and hence inhibits the decarboxylation. This then explains why only β-keto di- and tri-carboxylic acids decarboxylate easily while β- and α-keto-mono-carboxylic acids do not (cf. LX and LXI).

Steinberger and Westheimer (58) found that the decarboxylation of dimethyl oxaloacetic acid, LVI, was catalysed by heavy metal ions, while that of the monoester, LXII, was not.

$HOOC\text{-}CO\text{-}C(CH_3)_2COOH$ — LVI

$C_2H_5OOC.CO.C(CH_3)_2COOH$ — LXII

The following mechanism was suggested:-



LXIII LXIV LXV

The evidence for this proposed mechanism was as follows:-

1) The end-product was isolated and identified as α-keto iso valeric acid, LXV.

2) An enolic type intermediate was detected both spectroscopically and by bromine titration.

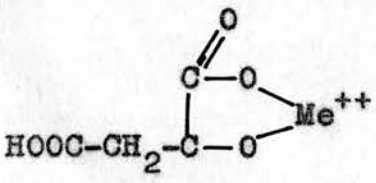
3) The monoester of the dimethyl oxaloacetic acid does not decarboxylate with heavy metal ions; the complex formation evidently takes place, if at all, between the carboxyl group and the β-keto group, so stabilising the molecule.

The significance of the charge on the metal ion in complex formation is well known. Gelles (128) studied the effect of para- and diamagnetic ions on the rate of decarboxylation of oxaloacetic acid to give pyruvic acid. He found that the paramagnetic rare earth Dy^{+++} increased the reaction rate by 20% more than did the very similar ion Y^{+++} which has the same association constant but is diamagnetic. The order of catalytic effect was

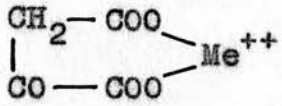
$$Lu^{+++} > Dy^{+++} > Y^{+++} > Gd^{+++} > La^{+++}$$

Expanding this work (129), a similar mechanism to that suggested by Steinberger and Westheimer was proposed. The rate coefficients were found to be in the order $Cu^{++} > Ni^{++} > Zn^{++} > CO^{++} > Mn^{++} > Ca^{++}$ and were, in fact, found to be proportional to the stability constants of the complex.

According to Steinberger and Westheimer the maximum rate of decarboxylation occurs at pH 4.5. Williams (130) suggested that the reason for this was the formation of two types of complex, one an α-keto-acid complex, LXVI, and the other a dicarboxylic acid complex, LXVII.

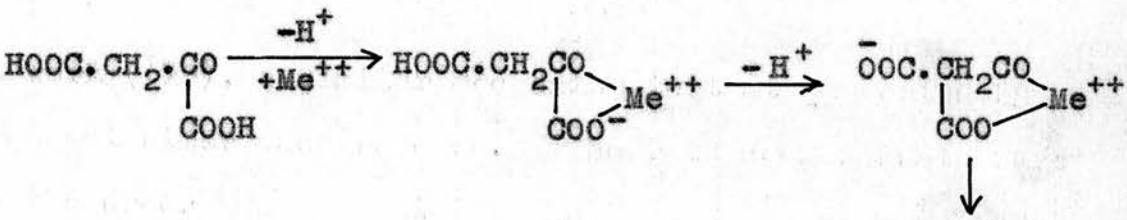


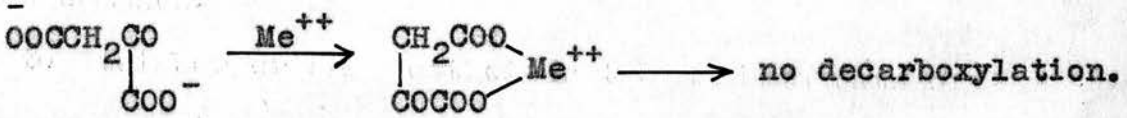
LXVI



LXVII

He investigated the stability constants of the Cu^{++} and Zn^{++} oxaloacetic acid complexes and compared the values with complexes of (a) a typical α-keto acid of type LXVI, e.g. pyruvic acid, and (b) a typical dicarboxylic acid. He found that the stability constants for Cu^{++} and Zn^{++} complexes with a dicarboxylate anion are, in general, higher than for pyruvic acid. From this he suggested a possible mechanism to explain the pH dependence of the decarboxylation:-

At low pH. 

At high pH. 

Complexing agents such as citrate and acetate ions reduce the catalytic activity of copper. These compete with the oxaloacetic acid for the Cu^{++} ions, and decrease the concentration of the chelate complex, LXVI. Pyridine also forms a complex with copper, but this is much weaker than the acid complex and so does not inhibit the reaction.

To avoid the catalytic oxidation by metals of natural compounds, e.g. Ascorbic acid, it should be possible to inactivate the metal by competitive complexing. Lück (131) found that iron, which

catalysed the enzymic decomposition of hydrogen peroxide, could be rendered inactive by complexing with phosphates. However, the activity was restored when ethylene diamine tetra-acetic acid (EDTA) or nitrilo-acetic acid (NTA) were added. The same effect was observed by Scaife (132) who found that a copper-glycine complex was a more effective oxidising agent for ascorbic acid than was copper alone.

The decarboxylation of carboxylic acids by anions has also been noted by various workers. Acetate ions have been found to catalyse the decomposition of dihydroxytartaric acid and also of oxaloacetic acid (133). Cyanide ions have been found to catalyse the decarboxylation of α-keto-carboxylic acids (134); when this method was applied to α-keto-D-gluconic acid, D-arabinose was detected by paper chromatography.

(ii) The metal catalysed formation of CO_2 from carbohydrate materials.

Nickerson (135) investigated the effect of various metal ions on the amount of CO_2 liberated from glucose in acid solution; the results are shown in table XI(a).

TABLE XI(a)

The effect of metal chlorides in 9% HCl on the evolution of CO_2 from glucose.

Salt	Salt concn. M.	% moles CO_2 per mole glucose in 5 hrs.
$FeCl_3$	0.5	65
$CuCl_2$	0.5	29
$SnCl_4$	0.5	1.6
$NiCl_2$	0.5	0.7
None	0	0.3

The rate of evolution of CO_2 was found to be proportional to the $FeCl_3$ concentration in 9% HCl. This strength of acid was used because it was found that, with 0.5 M. $FeCl_3$, the formation of dark-brown polymeric precipitates occurred at higher acid concentrations. The evolution of CO_2 was found to be approximately linear with time under these conditions, and also proportional to the weight of glucose taken. Nickerson suggested that there is an induction period before the evolution of CO_2 from glucose in the presence of iron, and also suggested the application of this method, using the standardised conditions for the determination of glucose in starches. Precautions have to be taken to ensure the purity of the sample used, although acetic acid, liberated from acetylated starches,

is not oxidised, and does not interfere. Fructose, under the same conditions, gives a much faster reaction, and a much greater yield; the rate of formation fell off exponentially. The curve for fructose, however, was obtained by a different method from the curves for glucose and sucrose.

Voss and Pfirschke (136) and Freudenberg, Gudjons, and Dumpert (137) found that mono- and polyuronic acids gave the theoretical yield of CO_2 on heating at 155-160° for 3-5 hrs. in 20M. zinc chloride solution of pH 2.5-3; furfural was also formed. The actual mechanism of the action of the zinc chloride is not very well understood. Probably the concentrated zinc chloride solution dehydrates the molecule to give an unsaturated carboxylic acid; this then decarboxylates, either thermally (66) or by a type of acid catalysed reaction (50).

Stutz (65) also used 20M. $ZnCl_2$ solution and found that monogalacturonic acid and glucurone decarboxylated much faster than substituted uronic acids. His results are shown in Table XI(b).

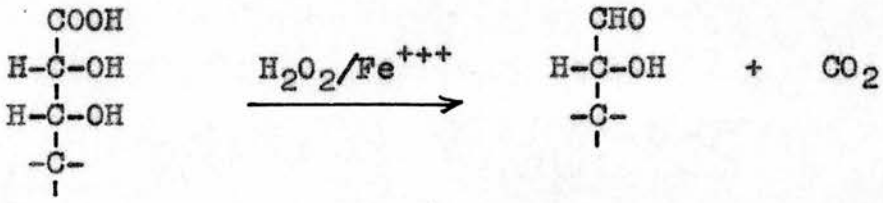
TABLE XI(b)

Decarboxylation of various uronic acid compounds in 20M. $ZnCl_2$ at 100°C for 4 hrs.

Compound	% moles CO_2 per mole
D-galacturonic acid	80
D-glucurone	60
4-O-methyl-α-methyl-D-glucuroniside amide	16
3-O-(β-D-△ 4,5-glucoseenuronic acid)-2-deoxy-2-acetyl-amino-D-glucose	24

It is seen from this table that the yields are not theoretical in 4 hrs. under these conditions. Graphical results given by Stutz show that polygalacturonic acid is decarboxylated very much slower than the monomer and at no time reached the theoretical value.

The oxidative decarboxylation of aldonic acids by the Ruff method (138) (139) has contributed to preparative carbohydrate chemistry. The pentose is formed from the aldonic acid by heating in the presence of ferric acetate and hydrogen peroxide.



Aldonic acid pentose

The yield of pentose is often small e.g. Ca-D-galactonate gives 17% D-lyxose, and Ca-D-gluconate gives 50% D-arabinose.

Zweifel and Deuel (140) (72) studied the decarboxylation of uronic acids by metal ions both in aqueous solution and also in pyridine. The CO_2 formed was estimated by absorption in baryta; nitrogen was used as a flow-gas. A very efficient condenser and scrubbers of phloroglucinol and silver nitrate were used.

Zweifel found that Al^{+++} was a better catalyst than Cu^{++} at pH 4 for the decarboxylation of D-galacturonic acid in water; arabinose was isolated from the solution. No investigation was

made using pure water without the addition of metal ions. In more strongly acid solutions, the rate of CO_2 formation decreased: this could be explained by a decrease in complex formation between the metal and uronic acid.

According to Zweifel, the catalytic action in aqueous solution is in the following order:-

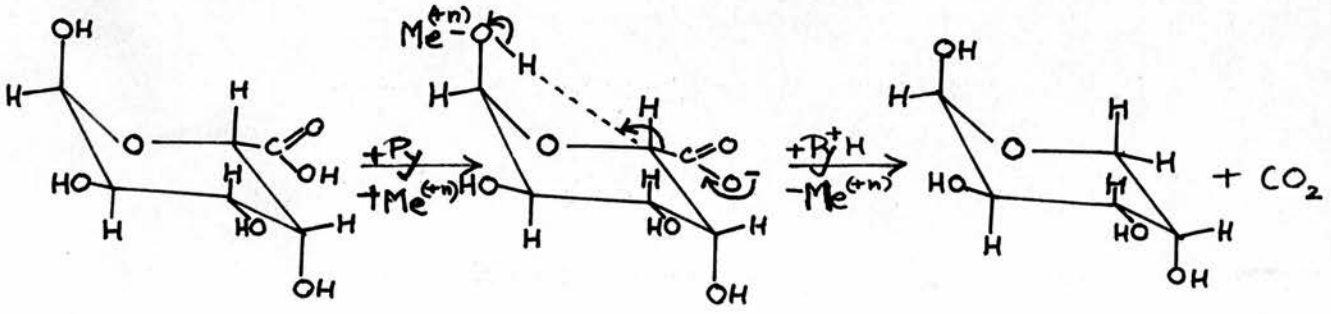
$$Pb^{++} > Al^{+++} > Ni^{++} > Zn^{++} > Cd^{++} > Mg^{++},$$

while for the action of metal acetates in pyridine solution the order was:-

$$Ni^{++} > Cu^{++} > Zn^{++} > Cd^{++}.$$

It was noted that Cu^{++} tended to oxidise the uronic acid with formation of a Cu_2O precipitate: the yield of CO_2 from galacturonic acid in pyridine with 0.99 moles nickel acetate present was practically theoretical in 120 min.

By comparing the amount of CO_2 formed from various substituted uronic acids, Zweifel concluded that carbon atom 1 must have an unsubstituted hydroxyl group for decarboxylation to be possible. On this basis, the following mechanism was suggested:-



The actual results found are summarized in the following tables.

TABLE XII(a)

Decarboxylation of galacturonic acid by 1 millimole of metal acetate in pyridine at 100°C in 120 mins.

Salt added	% moles CO_2 per mole uronic acid
Nickel acetate	93
Copper acetate	90
Zinc acetate	75
Manganese acetate	62
Cadmium acetate	10
Pyridine only	4

Table XII(a) shows that nickel acetate was the most effective catalyst, and so it was used in the decarboxylation of other related compounds.

TABLE XII(b)

Decarboxylation of uronic acids and related compounds in pyridine at 100°C with 1 m.mole nickel acetate present.

Uronic acid	Time of reaction (min.)	% moles CO_2 per mole uronic acid
Galacturonic acid	60	83
Galacturonic acid methyl ester	60	43
2-Methyl galacturonic acid	60	25
Glucurone	60	40
2,3,4,trimethyl galacturonic acid	60	20
" " " "	240	50

Mixed solvents were also used.

TABLE XII(c)

Decarboxylation of mono- and digalacturonic acid in a mixture of 20 ml. pyridine + 20 ml. dimethyl formamide at 90°C for 240 min. with 1 m.mole nickel acetate present.

Uronic acid	% moles CO_2 per mole uronic acid
Digalacturonic acid	45
Galacturonic acid	89

It is well known that pyridine also complexes with metals, so that in effect the uronic acid must compete with the pyridine for the metal. In table XII(d), K_1 and K_2 are the stability constants for one metal ion to one pyridine molecule and for one metal ion to two pyridine molecules respectively (144).

TABLE XII(d)

The stability of various metal complexes with pyridine.

Metal	log K_1	log K_2
Cd^{++}	1.27	0.8
Co^{++}	1.14	0.4
Cu^{++}	2.46	1.87
Ni^{++}	1.78	1.05
Zn^{++}	0.95	0.5
Hg^{++}	5.1	4.9

Gluconic acid also forms complexes with some metals (144). The values for log K_1 are based on the formation of a complex of the form 1 mole metal to 1 mole ligand.

TABLE XII(e)

The stability of various metal complexes with gluconic acid.

Metal	log K_1
Zn^{++}	1.70
Ca^{++}	1.21
Sr^{++}	1.00
Ba^{++}	0.95
Mg^{++}	0.70
Cu^{++}	18.29

In the case of Cu^{++}, the gluconic acid is regarded as H_2L, hence in this case the complex is extremely stable.

It is seen that a general relationship exists between the stability constants and the catalytic ability of the metals. No reference could be found to stability constants of metals with uronic acids.

The substances found to give very little decarboxylation had the C-1 hydroxyl group blocked, e.g. galactonic acid, mucic acid, saccharic acid, 2-furan carboxylic acid, tetrahydrofuran carboxylic acid, ascorbic acid, α-methyl galacturonic acid and

polygalacturonic acid. α-Keto glutaric acid, found to be stable in hot mineral acid, decarboxylated at 100°C in pyridine-dimethylformamide (20 ml.:20 ml.) giving 35 mole % CO_2 in 60 min.

On the basis of the above results Zweifel suggested that the metal formed a complex with the hydroxyl group on carbon 1 as in the mechanism shown above. The evidence put forward to support this can be summarised as follows:-

1) α-Methyl galacturonic acid and polygalacturonic acids, which do not decarboxylate, have the -OH group on carbon-1 blocked; when the -OH group on carbon-1 is free, decarboxylation does occur.

2) The straight chain acids, e.g. galactonic acid, do not decarboxylate, while the cyclic structure of galacturonic acid facilitates decarboxylation.

3) The formation of a complex by the metal with the ring oxygen atom does not cause decarboxylation; this is shown by the resistance to decarboxylation of tetrahydro-2-furan carboxylic acid.

In contrast to this are the following facts:-

1) The mechanism put forward has not been found in any other decarboxylation reaction.

2) The similarity to the "push-pull" mechanism suggested by Swain (145) is not valid since this mechanism takes place through the carbon chain and not between sterically adjacent groups as suggested in the Zweifel mechanism. However, this mechanism would be blocked if the -H on the -OH group was replaced by methyl; this, in fact, is found to be so.

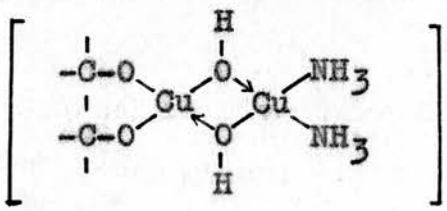
3) In pyridine the main product should be the appropriate pentose; only traces of pentose were, in fact, isolated.

4) It is unlikely that Ni^{++} and Cu^{++} will complex with only one hydroxyl group in a polyhydroxyl compound. Complexes of metals with sugars have been studied (146) (147) and the structures found. Reeves (148) used the formation of cuprammonium complexes as evidence for cis hydroxyls in sugars.

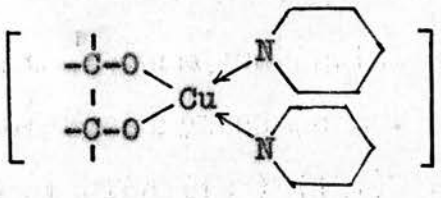
5) There is a very marked decrease in the rate of decarboxylation of 2-methyl- and 2,3,4,-trimethyl galacturonic acid as compared to unsubstituted galacturonic acid, suggesting that other hydroxyl positions affect the decarboxylation, as well as position 1. (Table XII(b)).

6) Since methyl groups tend to donate electrons as compared to hydrogen, the steric effect must predominate in this reaction.

7) The coordination number of copper is 4, and strong planar complexes are usually formed. The suggested complex has only one of the four coordination bonds of copper taken up: this is unlikely. In other complexes formed with polyhydroxy compounds the full planar coordinating ability of copper is displayed e.g. in the cuprammonium complex with glucose.



A possible structure for the complex in pyridine would be



Further work should, however, be carried out to ascertain the actual structure of the copper-uronic acid complex, and also the rates of decarboxylation of other uronic acids, both substituted and non-substituted, under similar conditions.

It was decided to investigate three possible reactions using the apparatus shown in Fig. 1.

1) The decarboxylation of various uronic acids by metals in pyridine in an attempt to repeat Zweifel's work;

2) The effect of metals on the decarboxylation of uronic acids in aqueous solution;

3) The decarboxylation of uronic acids in 19% (w/w) hydrochloric acid in the presence of metal ions.

EXPERIMENTAL

The apparatus described in Part I (p. 44) was used throughout.

L(-)-sorbose and many of the antioxidants and inorganic chemicals were supplied by B.D.H. Ltd. D(+)-galactose was supplied by T. Kerfoot and Co. Ltd., and D-galacturonic acid monohydrate by Roche Biochemicals Ltd.

EXPERIMENTAL RESULTS

(i) The catalytic effect of metal ions on the decarboxylation reaction in aqueous solution.

Initial experiments were carried out on galacturonic acid using pyridine in the presence of metal catalysts as the decarboxylating solution. Unfortunately, large and variable amounts of pyridine were found to come over, so introducing errors into the determination. It was therefore decided to use metal acetates in aqueous solution as catalyst. The results are shown in Table XIII(a).

TABLE XIII(a)

The decarboxylation of galacturonic acid monohydrate in pure water containing various metal acetates.

Conditions:-
1 milligram-atom of metal ion added as the salt.
Wt. of galacturonic acid monohydrate = 30 mg.
Vol. of water = 50 mls.
Results given as % moles CO_2 per mole uronic acid.

Salt added	None	Nickel Acetate	Zinc Acetate	Cobaltous Acetate	Magnesium Acetate	Barium Acetate	Calcium Acetate	Uranium Acetate	Thallous Acetate
Time (sec.)									
2000	1.5	11.5	26.4	19.0	3.2	1.8	2.5	12.2	2.0
5000	3.8	25.0	47.8	37.7	8.0	4.5	6.1	25.5	4.4
10000	7.8	41.7	67.2	58.2	15.5	8.8	11.6	40.2	9.1
20000	15.1	60.3	78.4	72.8	29.0	16.7	21.6	58.6	16.9
30000	21.9	68.8	82.6	77.2	40.4	24.2	30.4	70.6	24.0
50000	34.0	77.1	87.1	82.1	57.0	37.3	45.4	83.2	36.4
80000	50.1	82.7	91.3	86.2	73.6	54.6	63.3	92.5	52.0
00000	59.2	84.6	93.3	88.0	82.2	64.3	72.8	96.3	61.4
$(sec.^{-1}) \times 10^6$	6.6	88.8	184.0	138.0	15.9	7.9	10.5	69.1	8.0

From the table it is seen that the rate of decarboxylation decreases in the order:-

$$Zn^{++} > Co^{++} > Ni^{++} > UO_2^{++} > Mg^{++} > Ca^{++} > Tl^{+} > Ba^{++}.$$

Zn^{++} has, surprisingly, the most active acetate.

Cupric and mercuric acetates were found to give values very much higher than theoretical, indicating decomposition of the carbon chain, presumably through oxidation.

Basic aluminium acetate, basic ferric acetate, chromic acetate and lead acetate were all found to give incomplete decarboxylation. All these acetates gave precipitates, presumably of the hydroxide which removed some of the galacturonic acid from solution by complexing or co-precipitation.

Sodium acetate gave much the same decarboxylation curve as pure water. In no case was the decarboxylation totally complete, even after 100,000 sec.

TABLE XIII(b)

Decarboxylation of galacturonic acid monohydrate in pure water containing various metal salts.

1 mg. atom of metal ion added as salt, Vol. of water, 50 ml.

Wt. of galacturonic acid monohydrate = 30 mg.

Results in % moles CO_2 per mole uronic acid.

Salt added	Time (sec.)								k_1 (sec.$^{-1}$) x 10^6
	2000	5000	10000	20000	30000	50000	80000	100000	
None	1.5	3.8	7.8	15.1	21.9	34.0	50.1	59.2	6.6
Nickel chloride	2.5	5.7	10.7	20.5	29.5	44.9	63.3	72.7	9.5
Mercuric chloride	2.3	5.2	9.9	19.2	27.8	44.2	66.1	78.6	7.2
Zinc chloride	3.4	8.1	15.8	30.1	42.2	62.1	81.9	95.8	148.0
Lead chloride	3.0	7.0	13.1	24.8	35.7	53.9	74.5	84.5	11.6
Ferric sulphate	39.5	65.7	80.4	87.7	92.4	99.4	107.2	113.9	29.1

Sodium and calcium chlorides give the same curve as with water alone.

Zn^{++} is again the most efficient decarboxylating agent, the order for the chlorides being

$$Zn^{++} > Pb^{++} > Ni^{++} > Hg^{+}.$$

Cupric chloride and cupric sulphate both give the same curve and, along with ferric chloride, give amounts of CO_2 up to 50% greater than theoretical in 24 hr. The values for ferric sulphate are also greater

than theoretical after 80,000 sec., the Guggenheim plot being poor. In the case of the ferric salts, as opposed to the ferric acetate results (Table XIII(a)), the solution, after 24 hr. was colourless, showing that the iron had been removed as a complex by the products of decarboxylation; these were not investigated but would provide an interesting starting point for future work. Stannous chloride gave a white precipitate, (probably stannous hydroxide), and a low yield of carbon dioxide (cf. previous results for acetates which gave precipitates).

It is well known that borates complex very strongly with polyhydroxy compounds having cis-hydroxyl groups.

The catalytic effect of various metal borates on the decarboxylation of galacturonic acid monohydrate was investigated; the results are shown below.

TABLE XIII(c)

The decarboxylation of galacturonic acid monohydrate in pure water containing various metal borates.

Wt. of galacturonic acid monohydrate = 30 mg.
Vol. of water = 50 ml.
1 mg. atom of metal ion added as salt.
Results as % moles CO_2 per mole.

Salt added	Time (sec.)							
	2000	5000	10000	20000	30000	50000	80000	100000
None	1.5	3.8	7.8	15.1	21.9	34.0	50.1	59.2
Sodium Borate	7.7	17.2	29.4	40.6	44.6	48.8	52.6	54.7
Manganese Borate	18.0	27.0	34.0	41.8	47.2	53.9	60.7	63.8
Lead Borate	11.8	16.7	20.8	25.0	27.4	30.6	33.4	34.6

For all the borates it was found that the curves of % CO_2 versus time rose sharply initially, gradually flattening out at values much lower than theoretical. Although precipitation occurred with the heavy metals present it is considered that the divergence from stoichiometry is due to the complexing power of the borate. Despite the flattening of the curves there is still some decarboxylation; this may be due to the metal complexing with the galacturonic acid giving slow decarboxylation despite the overwhelming stability of the borate-uronic acid complex.

Zweifel and Deuel (72) found that polyuronides, in the presence of nickel acetate, did not decarboxylate in pyridine. Table XIII(d), however, shows the results obtained using nickel acetate in water.

TABLE XIII(d)

The decarboxylation of polyuronides in aqueous solutions of nickel acetate.
1 mg. atom of Ni^{++} added as nickel acetate.
Vol. of water = 50 ml.
Wt. of galacturonic acid monohydrate = 30 mg.
Results as % moles CO_2 per mole.

Uronic acid	Time (sec.)							
	2000	5000	10000	20000	30000	50000	80000	100000
Alginic acid	5.1	9.5	13.6	18.6	21.9	26.3	29.9	30.8
Trigalacturonic acid	11.6	21.5	30.9	40.6	46.1	51.3	55.0	56.5

Both these acids give an initial rapid evolution of CO_2 which decreases giving a flat curve of % CO_2 versus time. It is suggested that this is due to the initial catalytic action of the metal which then gradually complexes either with the product of decarboxylation or with the polyuronide, so inhibiting further decarboxylation. The values for pure water alone are given in Table IX(a) (p. 91).

(ii) The catalytic effect of metal ions on the decarboxylation reaction in 19% (w/w) HCl.

It was considered of interest to investigate the effect of traces of a suitable metal ion, likely to be found in natural polysaccharide materials, on the amount of CO_2 formed from non-uronic acid and uronic acid materials. Table XIII(e) shows the effect of ferric ions on the rate of formation of CO_2 from glucose.

TABLE XIII(e)

The effect of ferric ions on the yield of carbon dioxide from pure glucose in 19% (w/w) HCl.

Wt. of glucose, 30 mg. Vol. of acid = 50 ml.

Results as % moles CO_2 per mole sugar.

Parts per million Fe^{+++}	Time (sec.)							
	2000	6000	10000	20000	30000	50000	70000	100000
0	0.5	1.9	2.8	4.4	5.4	6.6	7.3	7.7
1	1.5	3.6	4.7	6.5	7.6	9.1	10.2	11.2
2	1.9	4.3	5.7	7.8	8.9	10.5	11.7	12.9
5	2.5	5.1	6.7	9.4	10.7	12.7	13.9	15.2
100	6.8	10.9	12.7	15.3	17.0	19.4	20.9	22.1
5000	25.1	61.4	68.7	78.6	85.0	94.2	101.0	108.7

The table shows that with the addition of a small amount (1 p.p.m.) of iron there is a rapid increase in rate of evolution of CO_2. This rapid initial increase is not maintained at higher iron concentrations although an increased evolution of CO_2 is given with increasing iron concentration.

Table XIII(f) shows the results for glucurone and galacturonic acid monohydrate as compared to glucose at $2\frac{1}{2}$ hr. as the reaction time. This is also the time recommended for the analytical determination (p.102). This shows that high results will be obtained if traces of metals are present in the uronic acid.

TABLE XIII(f)

The effect of ferric ion on the decarboxylation of uronic acids in 19% HCl.

Time of reaction = 9000 sec. ($2\frac{1}{2}$ hr.).

Results as % moles CO_2 per mole monomer.

p.p.m. Fe^{+++}	Glucurone	Galacturonic Acid Monohydrate	Glucose
0	97.0	96.0	2.6
1	100.2	97.6	4.5
5	101.4	98.1	6.3
100	102.4	100.5	12.3
500	105.2	103.0	16.7
1000	108.7	106.1	22.9
2000	116.0	112.2	32.8

It seems from Tables XIII(e) and XIII(f) that there is a great deal more overoxidation, proportionally, of glucose than of the uronic acids.

Further investigation, using polysaccharides containing uronic acids, should therefore be carried out to determine the actual extent of the possible error, and also the effect of other metals commonly found in the ash content of polysaccharide materials e.g. Mn^{++}; the present work has shown that errors arise in the decarboxylation method if parts per million quantities of iron are present.

(2) Antioxidants

Introduction

Antioxidants are widely used in the food industry in the United States and Canada and on a smaller scale in this country.

Early workers divided antioxidants into two main classes, primary inhibitors and acidic synergists. The primary inhibitors consisted mainly of o- and p- substituted phenols and aromatic amines. The synergists were substances, like citric and phosphoric acid, which had little or no action alone but enhanced the antioxidant activity of a primary inhibitor, mainly by deactivating traces of prooxidant metal.

Commonly used are n-propyl gallate, nordihydroguaiaretic acid, caffeic acid and various flavonoids, all of which can be obtained from natural sources.

Butylated hydroxy compounds of anisole and toluene are very stable and are very widely used in conjunction with metal inhibitors such as citric acid.

Aromatic amines have not been used nearly so widely in foods as in, for example, the rubber industry, because of their tendency to form coloured compounds, and the possibility of their acting as carcinogens.

The use of EDTA as a synergist in foodstuffs has been very strictly controlled because of possible subsequent complexing of metals in the body.

Usually a combination of antioxidants is most effective,

although it is possible that a low molecular weight complex will be a more potent oxidant than the metal itself (131) ~~e.g. iron bound with phosphoprotein is rendered inactive by the addition of the stronger complexing agents EDTA or NTA.~~

A study of the effects of added antioxidants on the amount of CO_2 liberated from uronic and non-uronic acid materials was undertaken in an attempt to find an antioxidant or complexing agent which would decrease the spurious CO_2 from non-uronic acid materials without interfering with the quantitative liberation of CO_2 from uronic acids.

RESULTS

The effect of 500 mg. of various antioxidants on the amount of CO_2 given off on boiling 180 mg. of (a) D-galactose and (b) L-sorbose in 19% HCl was estimated. The results are given below.

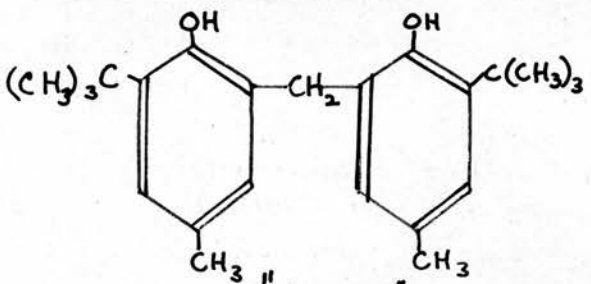
TABLE XIV(a)

The effect of antioxidants on the liberation of carbon dioxide from D-galactose in 19% (w/w) HCl.
Results as apparent % uronic acid anhydride (% U.A.A.).

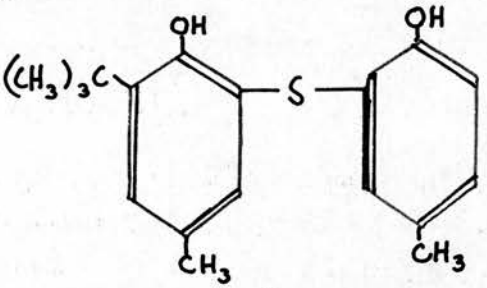
Antioxidant	Time (hrs.)					
	2	4	6	8	12	20
None	2.4	3.9	4.7	5.0	5.4	6.2
o-phenylene diamine	0.7	0.9	1.1	1.3	1.5	1.9
Pyrogallic acid	1.0	1.5	1.8	2.0	2.4	2.9
N-phenyl-2-naphthylamine	1.1	1.8	2.1	2.4	2.7	3.2
N-1-naphthyl-ethylene diamine di-hydrochloride	1.1	1.7	2.2	2.4	3.0	3.6
p-amino diphenylamine	1.6	2.3	2.6	2.9	3.4	3.9
Hydroquinone	1.2	2.0	2.4	2.9	3.4	3.0
N-N'-diphenyl-p-phenylene diamine	1.4	2.3	2.8	3.2	3.6	4.2
m-phenylene diamine	2.1	2.7	3.1	3.5	3.9	4.5
N-phenyl-1-naphthylamine	1.8	2.6	3.1	3.5	4.0	4.6
p-phenylene diamine	2.0	2.7	3.3	3.6	4.1	4.7

The results are expressed as apparent % U.A.A. to show how the extent of the positive contribution by sugars to the error in the determination of uronic acid content of a polymer can be decreased.

Other common antioxidants, e.g. n-propyl gallate, butylated hydroxy anisole, n-propyl-p-hydroxy benzoate, butylated hydroxy toluene, "CAO 6", and "CAO 5", were tested but all decomposed in the 19% HCl to give n-propyl chloride or tertiary butyl chloride; these react with $Ba(OH)_2$, giving rise to large errors, and so were not investigated further.



"CAO 5"



"CAO 6"

Similar results were obtained using L-sorbose.

TABLE XIV(b)

The effect of various antioxidants on the yield of carbon dioxide from L-sorbose in 19% (w/w) HCl.

Results as % U.A.A.

Antioxidant	Time (hrs.)					
	2	4	6	8	12	20
None	3.8	4.8	5.3	5.6	6.8	7.1
Pyrogallic Acid	0.8	1.1	1.3	1.5	1.8	2.3
o-Phenylene diamine	1.0	1.4	1.7	1.9	2.2	2.5
N-phenyl-2-naphthylamine	1.9	2.3	2.6	2.9	3.3	3.7
Hydroquinone	1.8	2.3	2.6	2.9	3.4	3.8
N-1-naphthyl ethylene diamine dihydrochloride	2.0	2.5	2.9	3.2	3.6	4.1
p-Amino diphenylamine	1.9	2.5	3.0	3.3	3.8	4.3
N-N'-diphenyl-p-phenylene diamine	2.1	2.8	3.3	3.5	4.0	4.4
N-phenyl-1-naphthylamine	2.4	3.1	3.5	3.8	4.3	4.8
m-Phenylene diamine	2.9	3.7	4.1	4.4	4.9	5.6
p-Phenylene diamine	3.0	3.8	4.4	4.7	5.3	6.1

A comparison of Tables XIV(a) and (b) shows that pyrogallic acid and o-phenylene diamine give the maximum decrease in CO_2 liberation in the case of both D-galactose and L-sorbose.

The above antioxidants were then added to galacturonic acid monohydrate in 19% HCl in the hope that one of them would suppress the side reaction without suppression of CO_2 yield from the carboxyl group.

TABLE XIV(c)

The effect of antioxidants on the decarboxylation of galacturonic acid monohydrate in 19% (w/w) HCl.

Results as % U.A.A.

Antioxidant	Time (hr.)				
	1	2	4	8	20
None	76.5	78.8	81.8	85.5	88.8
Decrease in CO_2 desired (approx.) (see Table XIV(a))		-2.4	-3.9	-5.0	-6.2
Pyrogallic Acid	35.0	41.0	47.0	53.0	58.0
o-Phenylene diamine	70.1	72.1	74.0	75.3	76.5
N-phenyl-1-naphthylamine	71.0	72.8	75.1	76.8	77.3
N-phenyl-2-naphthylamine	72.3	73.1	75.4	77.1	77.4
p-Amino diphenylamine	73.2	74.6	76.3	78.7	80.1
N-1-naphthyl ethylene diamine dihydrochloride	75.1	76.0	77.0	80.4	85.0

It seems, therefore, from these results, that of these antioxidants only the N-1-naphthyl ethylene diamine dihydrochloride suppresses the extraneous CO_2 formation from sugars without also suppressing the desired decarboxylation. The mechanism of the antioxidant action is not understood, although these results offer encouragement for future supplementary studies.

PART III

The formation of carbon dioxide from non-uronic acid carbohydrate materials and related compounds.

INTRODUCTION

As indicated in Part I, the determination of the uronic acid content of a polysaccharide can be carried out by estimation of the CO_2 liberated by boiling, with either 12% or 19% HCl, for a time usually taken to be that required to give the theoretical yield of CO_2 from a pure uronic acid standard, e.g. pure glucurone or galacturonic acid. If the mixture is boiled for a longer period, CO_2 continues to be liberated from side reactions even though all carboxyl groups, present in the original starting material, must have been eliminated; such side reactions clearly run concurrently throughout the decarboxylation period.

If the elimination of CO_2 from the carboxyl group of a hexuronic acid is the first step, the resulting product will be a pentose. Various workers have investigated the amount of CO_2 eliminated from such non-uronic acid carbohydrates and related materials. Franken (149) found that the appropriate pentose was formed when uronic acids were treated with steam under pressure. In acid solution, however, only furfural could be isolated: this indicated that further decomposition of the initially formed pentose must have taken place.

Whilst most authors were content to report the time required for complete decarboxylation to the theoretical yield, Conrad (100) appears to have been the first to report values of over 100% for uronic acid content. Comparing the % uronic acid found using various strengths of acid after 16 hr. he found that 12% HCl gave 101.1% and 18% HCl gave 102.1% uronic acid for a pure pectin.

Norman and Martin (77), also working on pectins, found that other naturally occurring carboxylic acids, e.g. oxalic and lactic acids, gave rise to small amounts, and pyruvic acid to larger amounts of CO_2.

Guanzon and Sandstrom (150), using 12% HCl, suggested a correction in the % CO_2 from uronic acid materials of 0.45% of the weight of carbohydrate, to allow for the CO_2 from non-uronic acid sources.

The first real attempt to compare the CO_2 from various individual sugars was made by Hirst and co-workers (151) who found, using 12% HCl, that rhamnose gave the highest yield, while mannitol gave no CO_2. Norman (152) repeated some of this work, and also measured the rate of evolution of CO_2; he found that the rate for uronic acids was much greater than for non-uronic acid materials.

Whistler, Martin and Harris (80) found that the evolution of CO_2 from glucose was proportional to time in various strengths of acid.

While McCready and co-workers (92) found that 19% HCl and 12% HCl both gave the same amount of CO_2 from glucose, this has not been confirmed by other workers (80) (86).

Letzig (86), investigating the uronic acid content of all kinds of foodstuffs, found that 19% HCl definitely increased the CO_2 yield from non-uronic acid materials as compared with 12% HCl.

Tracey (84) decarboxylated a large variety of compounds in a sealed tube in 12% HCl at 110°C. and compared the results with those of previous workers.

Machida (71) determined the rates of evolution of CO_2 from non-uronic acid materials and also put forward a mechanism for the uronic acid decarboxylation. The actual kinetics of the evolution of CO_2 from individual non-uronic carbohydrates were determined by Huber (70) who found the rate constants assuming a zero-order reaction. The present work, however, shows that the validity of the assumption that the CO_2 liberation is of zero order is doubtful. The rate constants found by Huber show a distinct lack of constancy. In addition, the straight lines of Whistler and co-workers (80) are slightly curved at higher acid concentrations, indicating a first-order reaction; mucic acid, glucono-lactone and saccharic acid were also decarboxylated and the rate constants calculated on the assumption that the reactions were first order.

Perlin (66) found that ascorbic acid, mucic acid, and gluconic acid all gave CO_2 when heated under conditions which he claimed gave theoretical yields of CO_2 from uronic acids.

EXPERIMENTAL RESULTS

While some authors (84) had investigated the amount of CO_2 liberated in a given time, others (70) had found the complete kinetic curves for a small number of non-uronic acid carbohydrate materials. If decarboxylation followed a zero order reaction then a straight line plot of % CO_2 versus time would be given indicating that CO_2 would be liberated continuously with time.

An accurate investigation of the amount of CO_2 given off from non-uronic acid materials seemed desirable; it would give an accurate basis for correction of the apparent uronic acid content of polysaccharides of known constitution, as found by decarboxylation. Consequently the values summarised in Table XV(a) were found. For compounds which gave only small amounts of carbon dioxide 180 mg. samples were used; 30 mg. being used for those compounds which gave high yields of CO_2. Since the molecular weights vary from compound to compound, a direct comparison can only be made if the results are expressed as % moles CO_2 per mole.

TABLE XV(a)

The liberation of CO_2 from various non-uronic acid materials likely to interfere in uronic acid determinations in 19% (w/w) HCl.

Results as % moles CO_2 per mole of monomer.

Substance	Time (sec.)					
	3000	6000	9000	20000	40000	80000
D-glucose	0.98	1.57	1.94	2.62	3.14	3.64
D-galactose	1.40	2.23	2.87	4.29	5.48	6.54
D-glucoheptose	2.42	3.88	4.96	7.51	10.65	14.78
D-mannose	2.12	3.00	3.51	4.41	5.16	6.02
Chloralose	0.40	0.80	1.16	2.15	3.26	4.38
L-sorbose	2.80	3.80	4.40	5.54	6.53	7.60
D-fructose	1.57	1.99	2.26	2.93	3.71	4.55
L-arabinose	0.88	1.40	1.80	2.65	3.47	4.27
D-xylose	1.05	1.41	1.65	2.17	2.80	3.48
D-arabinose	0.86	1.40	1.78	2.60	3.37	4.23
L-xylose	0.41	0.68	0.88	1.36	1.91	2.56
D-lyxose	1.50	2.05	2.44	3.38	4.34	5.30
D-ribose	1.22	1.64	1.91	2.60	3.42	4.18
D-erythrose	2.17	2.83	3.28	4.27	5.23	6.09
DL-glyceraldehyde	0.20	0.34	0.44	0.65	0.86	1.06
Meso-inositol	0.10	0.18	0.25	0.45	0.68	0.83
Mannitol	0.16	0.36	0.39	0.48	0.56	0.61
Sorbitol	0.10	0.16	0.22	0.43	0.66	0.82
Dulcitol	0.08	0.13	0.18	0.32	0.46	0.54
Ribitol	0.20	0.36	0.47	0.79	1.26	1.93
Erythritol	0.02	0.05	0.07	0.13	0.22	0.33

TABLE XV(a) (Continued)

Substance	Time (sec.)					
	3000	6000	9000	20000	40000	80000
L-fucose	1.15	2.06	2.82	4.28	5.40	6.50
2-Deoxy-D-glucose	0.43	0.78	1.09	1.98	3.00	4.14
L-rhamnose	1.92	2.75	3.24	4.08	4.82	5.78
2-Deoxy-D-galactose	0.42	0.79	1.11	2.03	3.20	4.43
Digitoxose 2,6-dideoxy-D-allose	0.31	0.49	0.65	1.11	1.85	2.79
Sucrose	1.54	2.19	2.60	3.54	4.64	5.82
Cellobiose	0.53	0.99	1.34	1.95	2.32	2.57
Melibiose	0.65	1.18	1.60	2.56	3.28	3.85
Lactose	0.70	1.24	1.67	2.66	3.55	4.39
Maltose	0.56	0.96	1.27	1.82	2.11	2.32
Trehalose	0.64	1.28	1.75	2.53	3.15	4.00
Turanose	1.28	1.91	2.22	2.68	3.04	3.43
Raffinose	1.03	1.81	2.31	3.30	4.00	4.71
Melezitose	0.88	1.29	1.60	2.18	2.60	2.88
α-D-glucoheptono-γ-lactone	2.9	5.6	8.1	15.7	26.8	40.7
Ca-glucoheptonate	6.7	11.6	15.8	27.5	41.7	56.1
Galaheptono-γ-lactone	2.5	4.9	7.3	15.5	28.3	47.9
D-galactonic acid-γ-lactone	0.78	1.61	2.50	5.56	11.12	21.62
Gluconic acid-δ-lactone	3.5	6.8	10.0	21.3	37.7	53.5
Gulonic acid	2.3	4.8	7.2	15.9	30.6	54.0
Ca-L-idonate	6.2	11.7	17.4	34.6	53.9	66.5
L-gulonic acid-γ-lactone	4.0	7.9	11.7	24.1	42.9	64.8
2-Keto-gulonic acid	61.8	65.0	65.8	67.7	70.4	73.8
L-arabonic acid-γ-lactone	0.76	1.54	2.33	5.24	10.53	20.43
D-ribonic acid-γ-lactone	4.3	5.3	6.3	9.9	16.7	29.0
Mucic acid	1.2	2.5	3.7	8.4	16.8	32.8
Glucosamine hydrochloride	0.18	0.31	0.40	0.67	1.12	1.77
N-methyl-1-D-glucamine	0.19	0.35	0.48	0.84	1.22	1.66
Glucosaminic acid	16.2	30.1	41.0	62.1	72.5	78.3

TABLE XV(a) (Continued)

Substance	Time (sec.)					
	3000	6000	9000	20000	40000	80000
Reductone	2.00	2.85	3.29	3.99	5.67	6.46
Kojic acid	0.65	1.29	1.89	4.18	8.30	16.11
Reductic acid	0.05	0.10	0.14	0.25	0.39	0.58
Furoic acid	74.8	92.1	93.3	95.6	98.6	101.4
5-Hydroxy methyl furfural	0.94	1.23	1.40	1.81	2.28	2.95
Meconic acid	74.1	78.5	82.4	94.1	112.5	138.4
Coumalic acid	167.4	170.3	172.0	175.2	178.0	180.0
Tartronic acid	44.6	61.1	72.7	84.9	86.7	87.2
Chelidonic acid	2.7	5.1	7.3	13.8	22.2	27.4
5-Carboxy furoic acid	2.15	4.25	6.2	12.2	19.7	26.0
Muconic acid	2.8	5.6	8.4	17.7	32.9	57.9
Maltol	0.76	1.42	2.00	3.72	5.82	88.2
α-Keto glutaric acid	4.4	8.96	13.22	27.38	44.94	56.80
β-Keto adipic acid	1.75	2.41	2.82	3.81	5.01	6.58
Citric acid	0.35	0.55	0.69	1.03	1.44	2.00
Dihydroxy acetone	0.06	0.12	0.16	0.31	0.48	0.61
p-Amino benzoic acid	1.10	2.20	3.23	7.0	12.62	20.22
Quinic acid	0.10	0.19	0.27	0.50	0.82	1.18
Lævulinic acid	0.16	0.25	0.31	0.44	0.60	0.75
Glycerol	0.07	0.14	0.19	0.31	0.43	0.54
Glycolaldehyde	1.11	1.41	1.58	1.96	2.45	3.21
Ethylene glycol	0.08	0.15	0.20	0.30	0.38	0.54
Methyl glyoxalate	0.30	0.51	0.67	1.03	1.47	1.90
Sodium glyoxalate	0.77	1.10	1.33	1.96	2.95	4.55
Pyruvic aldehyde	1.10	1.80	2.30	3.60	5.22	7.16
Diglycollic acid	0.43	0.68	0.84	1.19	1.48	1.80
Itaconic acid	1.3	2.5	3.7	7.4	11.9	16.2
Aconitic acid	1.78	2.68	3.35	5.16	7.57	11.8

TABLE XV(a) (Continued)

Substance	Time (sec.)					
	3000	6000	9000	20000	40000	80000
Allantoin	8.5	16.0	23.3	47.9	86.8	136.0
Alloxantin	81.4	116.7	139.6	184.3	225.4	270.0
Alloxan	40.0	50.0	57.0	75.0	96.9	123.4
Hypoxanthine	17.8	33.7	45.9	76.6	90.4	94.9
Hydantoin	6.8	12.4	17.6	32.0	47.9	67.2
Hydantoic acid	10.2	18.4	25.3	45.0	63.7	76.0
Xanthine	0.91	1.80	2.41	5.41	10.8	21.0
Orotic acid	2.4	4.12	5.41	7.21	9.11	12.15

Related compounds are, in general, grouped together.

It is seen that all the sugars liberate some CO_2. Non-uronic carbohydrate acids, with no keto groups in the chain, liberate CO_2 much faster than the sugars but much slower than the uronic acids. A plot of % CO_2 versus time for these acids is practically a straight line initially with a gradual "tailing off" as for a first-order curve. These substances would tend to interfere with a uronic acid determination more than the sugars; the maximum value being for the rarer acids such as Ca-L-idonate and Ca-glucoheptonate. These were, however, commercial samples and may have contained a trace of the related uronic acid. Glucosaminic acid, which has not been found in polysaccharides to date, would also interfere to a large extent.

Various oxygen heterocyclic carboxylic acids and related compounds were also decarboxylated. The substitution of furoic acid to give 5-carboxy furoic acid decreases the rate of

decarboxylation very markedly. Substances which could be formed as intermediates or as decomposition products were also examined as were various naturally occurring nitrogen heterocyclics; some of the latter gave rise to large amounts of carbon dioxide.

Table XV(b) also summarises the values obtained in this work and compares the values with those obtained by previous workers. Since the % uronic acid in a polysaccharide is always calculated as % uronic acid anhydride, the values given in this table represent the apparent uronic acid content which would be obtained in a uronic acid determination. These, then, are the values which must be subtracted from a uronic acid value obtained from a polysaccharide if the composition is known. With the exception of McCready, Swenson and Maclay (92), who used 19% HCl, and Letzig (86), who used both 12% and 19%, all the previous workers give values obtained in 12% HCl. The compounds tested are divided into two groups, the first those (giving a value of over 5% U.A.A.) which would interfere seriously with a uronic acid determination of a polymeric natural product, and those giving less than 5% U.A.A. error; this latter includes all the common sugars. Very few polymeric materisls were analysed, mainly because of doubts about the absence or otherwise of uronic acid residues in these substances. In general it seems that polymeric substances, thought to contain no uronic acid groups, do give higher values than calculated on the basis of the additive contributions from their sugar components. Values for various sugars quoted by Huber (70) are all very much higher than those obtained by previous workers and are considered to be somewhat suspect.

TABLE XV(b)

A comparison of values found by various workers for the CO_2 yield from substances likely to interfere in uronic acid determinations.

Substance % U.A.A. > 5%	Yield of CO_2 expressed as % U.A.A.	
	Present work	Other Authors
Allantoin	25.9	30(13)
Alloxan	70.7	35.6, 39.2(13)
Alloxantin	85.9	
Ascorbic acid	99.4	68.4, 74.7(13); 99.0(15)
5-carboxy furoic acid	7.0	
Chelidonic acid	6.9	
Coumalic acid	216.0	
Furoic acid	146.6	149.2(17)
D-galaheptono-γ-lactone	6.2	
Ca-glucoheptonate	11.5	
D-glucoheptono-γ-lactone	6.9	
Gluconic acid-δ-lactone	9.9	8.2(14)
Glucosaminic acid	37.0	
Gulonic acid	6.5	
L-gulonic acid-γ-lactone	11.6	
Hydantoic acid	37.7	
Hydantoin	31.0	
Hypoxanthine	59.4	41.2(13)
Ca-L-idonate	13.0	
Isoascorbic acid	98.1	
Itaconic acid	5.0	
α-Keto-glutaric acid	16.0	
2-Keto-gulonic acid	59.8	
Meconic acid	72.4	
Muconic acid	10.4	
Orotic acid	6.1	
Pyruvic acid	14.2	18.8(13); 21.6(2)
Pyruvic aldehyde	5.6	

TABLE XV(b) (Contd.)

Substance % U.A.A. 5%	Yield of CO_2 expressed as % U.A.A.	
	Present work	Other Authors
Reductone	6.6	
D(+)ribonic acid-γ-lactone	7.5	
Tartronic acid	106.6	
Aconitic acid	5.4	
<5% p-Amino benzoic acid	4.2	15.3(13)
L(+)arabinose	2.1	2.4(13); 1.9(8); 2.8(11)
D(-)arabinose	2.1	
L(+)arabonic acid-γ-lactone	2.8	
Cellobiose	1.4	
Cellulose	0.81	1.2(13); 1.8(7); 0.68(8); 0.64(9); 0.81(14)
Chloralose	0.66	
Citric acid	0.63	0.8(13); 0.0(6)(7)
2-Deoxy-D-glucose	1.2	
2-Deoxy-D-galactose	1.2	
Digitoxose	0.77	
Diglycollic acid	1.1	
Dihydroxy acetone	0.31	
Dulcitol	0.17	
Erythritol	0.11	0.4(13)
D(-)erythrose	4.8	
Ethylene glycol	0.57	
D-fructose	2.2	2.2(8); 3.2(7); 2.4(9); 2.2(15); 1.5(16)
L(-)fucose	3.0	4.0(13)
D(-)galactonic acid-γ-lactone	2.5	
D(+)galactose	2.8	2.0(13)
Gelatin		c. 1.0(11); 0.0(15)
D-glucoheptose	4.2	
Glucosamine hydrochloride	0.33	0.4(13)

TABLE XV(b) (Contd.)

Substance % U.A.A. 5%	Yield of CO_2 expressed as % U.A.A.	
	Present work	Other Authors
D(+)glucose	1.9	2.8(13); 0.72(4); 2.9(6)(7); 1.6(8); 0.76(9); 1.0(10); 1.2(14)
DL-glyceraldehyde	0.86	
Glycerol	0.36	
Glycolaldehyde	4.6	
5-Hydroxy methyl furfural	1.96	
meso-inositol	0.24	0.4(13)
Inulin	-	3.4(13); 2.4(8); c. 1.0(11)
β-keto adipic acid	3.1	
Kojic acid	2.34	
Lactic acid	-	1.2(17); 0.32(2); 0.0(15); 0.3(18)
Lactose	1.63	3.1(15); 0.6(16)
Lævulinic acid	0.47	0.8(13); 0.4(17)
D(-)lyxose	2.9	
Maltol	2.8	
Maltose	1.31	1.9(15); 0.4(16); 1.2(8); 1.2(14)
Mannitol	0.37	0.0(8)
D(+)mannose	3.5	2.2(8)
Melibiose	1.6	2.8(13)
Melezitose	1.6	2.8(13)
N-methyl-1-D-glucamine	0.43	
Methyl glyoxalate	1.3	
Mucic acid	3.1	1.2(13); 3.6(17)
Oxalic acid	4.3	1.2(2); 3.2(3) 8.0(6)(7); 5.0(14)
Quinic acid	0.25	
Raffinose	2.1	

TABLE XV(b) (Contd.)

Substance % U.A.A. 5%	Yield of CO_2 expressed as % U.A.A.	
	Present work	Other Authors
Reductic acid	0.21	
L(+)rhamnose	3.5	3.6(8)
Ribitol	0.54	
D(-)ribose	2.2	2.4(13)
Sodium glyoxalate	2.4	
Sorbitol	0.21	
L(-)sorbose	4.3	
Sucrose	2.7	2.2(15); 0.9(16); 3.0(7); 3.1(6); 2.1(8); 0.96(9); c. 1.0 (11)
Trehalose	1.6	2.0(13)
Turanose	2.3	
Xanthine	2.8	
D(+)xylose	1.9	2.4(13); 1.6(8); 5.6(14)
L(-)xylose	1.0	

The numbers in brackets indicate the authors, as shown below.

		Reference in main bibliography
(1)	Nanji, Paton and Ling (1925)	(74)
(2)	Norman and Martin (1930)	(77)
(3)	Link (1931)	(153)
(4)	Anderson (1931)	(154)
(5)	Spoehr and Milner (1935)	(155)
(6)	Colin and Lemoyne (1938)	(156)
(7)	Colin and Lemoyne (1938)	(157)
(8)	Campbell, Hirst and Young (1938)	(151)
(9)	Norman (1939)	(152)
(10)	Whistler, Martin and Harris (1940)	(80)
(11)	McCready, Swenson and Maclay (1946)	(92)
(12)	Fuller, Bartholomew and Norman (1947)	(158)

		Reference in main bibliography
(13)	Tracey (1948)	(84)
(14)	Taylor, Fowler, McGee and Kenyon (1947)	(83)
(15)	Letzig (1950) (19% HCl)	(86)
(16)	Letzig (1950) (12% HCl)	(86)
(17)	Machida (1955)	(71)

It can be seen that a number of the substances investigated have not been tested before. In general, the values found in this work using 19% acid are lower than the values found by other workers, the majority of whom used 12% acid.

In the case of substances containing a carboxyl group, the source of the CO_2 evolved is easily explained. In the case of the sugars and non-carboxylic acid compounds, however, the source of the CO_2 is open to conjecture. No analysis was carried out on the polymeric materials formed in the acid decarboxylation. It must be presumed, therefore, that the carbon dioxide is formed either during the condensation of the dehydrated sugar chains to form polymers, or by slow, and possibly incomplete, oxidative decomposition of the sugars themselves. The use throughout of oxygen-free nitrogen may have minimised the latter possibility. Further investigation of the nature of the polymeric material might lead to some conclusion on this aspect of the work.

It is seen then that a number of substances present in plant, animal or soil material may interfere to a marked extent in uronic acid determinations. Consequently, in the use of this method of estimation, allowance must be made for the extraneous CO_2 from non-uronic acid sources.

PART IV

The gaseous products liberated from naturally occurring uronic acid and non-uronic acid materials in boiling 19% HCl

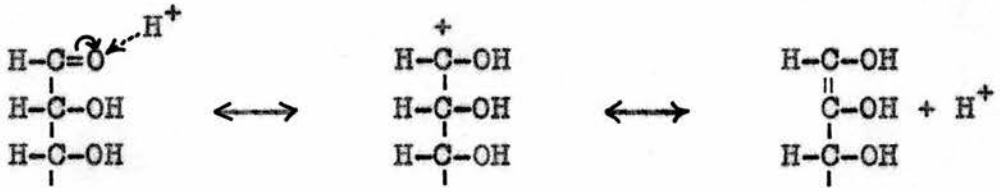
INTRODUCTION.

Previous workers have analysed the acid decomposition products from uronic acids and other carbohydrate materials. In most cases, investigations have been made in the liquid phase for products other than CO_2. It is well known that furfural, 5-hydroxymethyl furfural and 5-methyl furfural are produced from pentoses, hexoses and 6-deoxy sugars respectively; uronic acids also give large amounts of furfural.

1) The dehydration of aldoses and ketoses.

Aldoses and ketoses dehydrate faster than the corresponding polyols in acid media; their increased lability in acid solution can be ascribed to the carbonyl group.

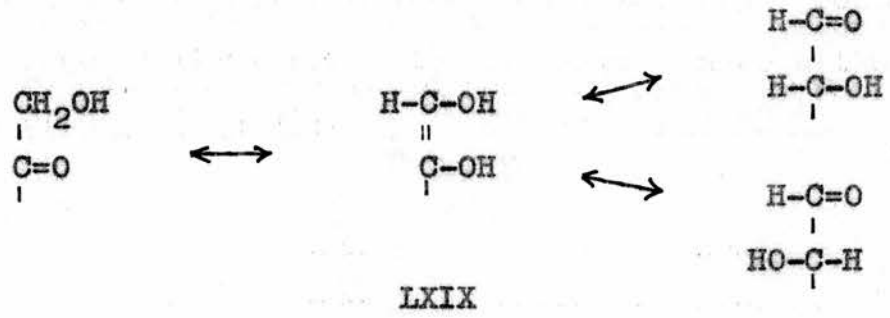
Free aldehyde and keto groups can undergo the following reaction (160)(69) (161):-



LXVIII

A proton from the solutions attacks the nucleophilic oxygen of the carbonyl group giving a positive charge on the carbonyl carbon atom so facilitating the release of a proton from the α-carbon atom to give the enediol, LXVIII. In pyranose and furanose aldoses and ketoses the enediol is formed by opening of the hemi-acetal ring. Evidence has been found by U.-V. spectroscopy

which suggests that glucose exists in the aldehydic form in a wide range of acid concentrations, e.g. from cold 0.001N.HCl (162) to cold 50% H_2SO_4 (163). The solution absorbs at 280-285 mμ, and this particular band disappears on neutralisation of the solution. Neutral fructose solution (164), in contrast to neutral glucose solution (165), absorbs in the U.-V. This evidence for enediol formation in aldose and ketose solutions is, however, only indirect. The epimer sugars can be obtained by heating with acetic acid (pH 2.8) under pressure (166). The epimerisation in acid is considered to be analogous to that in alkali, with ring opening and enediol formation; the enediol being very unstable.



LXIX

The following factors can, however, contribute to the stability of the enol form.

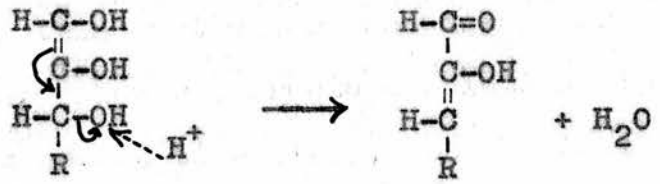
a) Substitution increases the proton mobility on the carbon atom of the keto form more than on the oxygen atom of the enol form.

b) Conjugation of the double bond of the enol form.

c) Shielding by substitution and chelation of the -OH group of the enol.

Epimerisation of the aldoses and ketoses, under more strongly

acid conditions, suffers competition from the elimination of a neighbouring -OH group in the β-position (166)



LXX LXXI

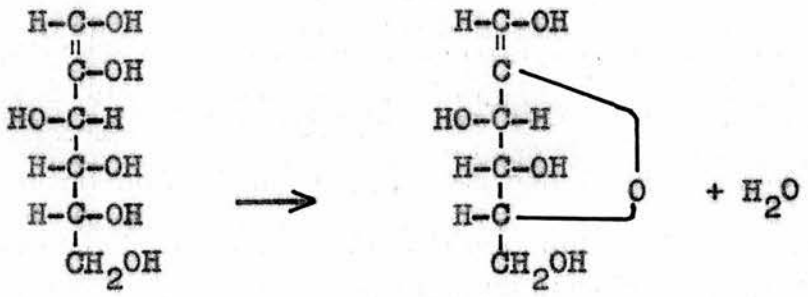
This elimination is due to the increase in the concentration of the H_3O^+ ion.

Wolfrom and co-workers (166) found that on heating a dilute glucose solution at pH 4.3 a product was formed which selectively absorbed at 228 mμ. They suggested that this absorption was due to an acyclic enediol, e.g. an enediol form of glucose (such as LXX above where R = $C_5H_6O_3$). They found that the absorption at 228 mμ rose to a maximum after 3.5 hrs. boiling and could not be due to hydroxymethyl furfural, which is the final product of the reaction with an absorption maximum at 285 mu ($\log \epsilon \approx 4$). After longer boiling, or by using solutions of greater acidity, the typical hydroxymethyl furfural peak was obtained.

With pentoses, the first steps of acid reaction consist of enediol formation and water elimination (167).

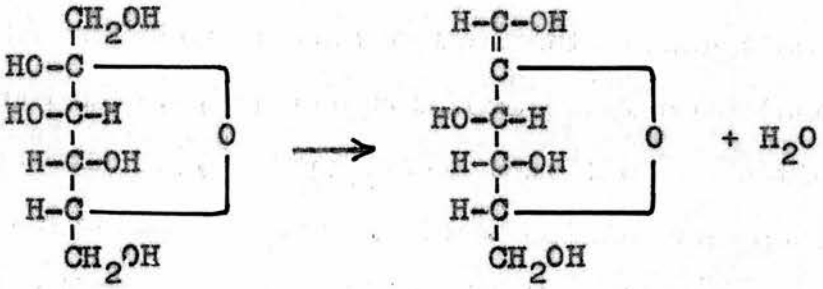
Haworth and Jones (168) proposed an initial enediol formation as the first step in the dehydration of glucopyranose, the first molecule of water being eliminated by ring closure between

C_2 and C_5.



LXXI LXXII

In the case of fructose the proposed first step was the elimination of water between C_1 and C_2.

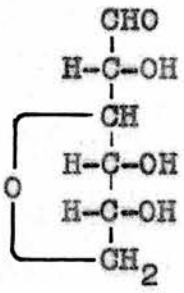


LXXIII LXXIV

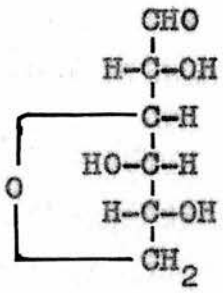
The enol, LXXIV, is the same for both glucose and fructose.

Various mechanisms have been put forward for the elimination of the first molecule of water from aldoses and ketoses; it is reasonable to assume that the steps of dehydration are the same for both aldoses and ketoses, i.e. (a) ring opening (b) enediol formation and water elimination in the position adjacent to the enediol.

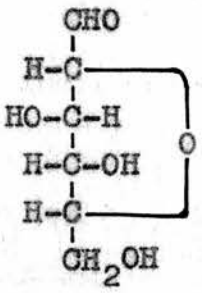
Very little keto-form occurs in dilute solutions of keto-furanoses and of certain anhydro sugars, e.g. 3,6-anhydro-D-glucose, LXXV, (169), 3,6-anhydro-D-galactose, LXXVI, (170), 2,5-anhydro-D-mannose, LXXVII, and 2,5-anhydro-L-arabinose, LXXVIII, (171) (172), all exist in the aldehydic form in water or $0.01\overline{N}.H_2SO_4$.



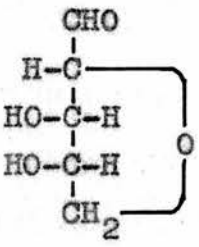
LXXV



LXXVI

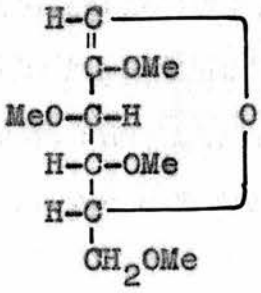


LXXVII

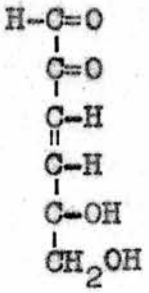


LXXVIII

Further steps in the dehydration of aldoses and ketoses are purely speculative; with one exception, none of the possible intermediates have been isolated. Tetramethyl-1,2-glucoseen, LXXIX, gives a good yield of 5-methoxy methyl furfural in 3N.HCl at room temperature (173). On treatment with phenyl-hydrazine the phenylosazone of the unsaturated osone, LXXX, was isolated.

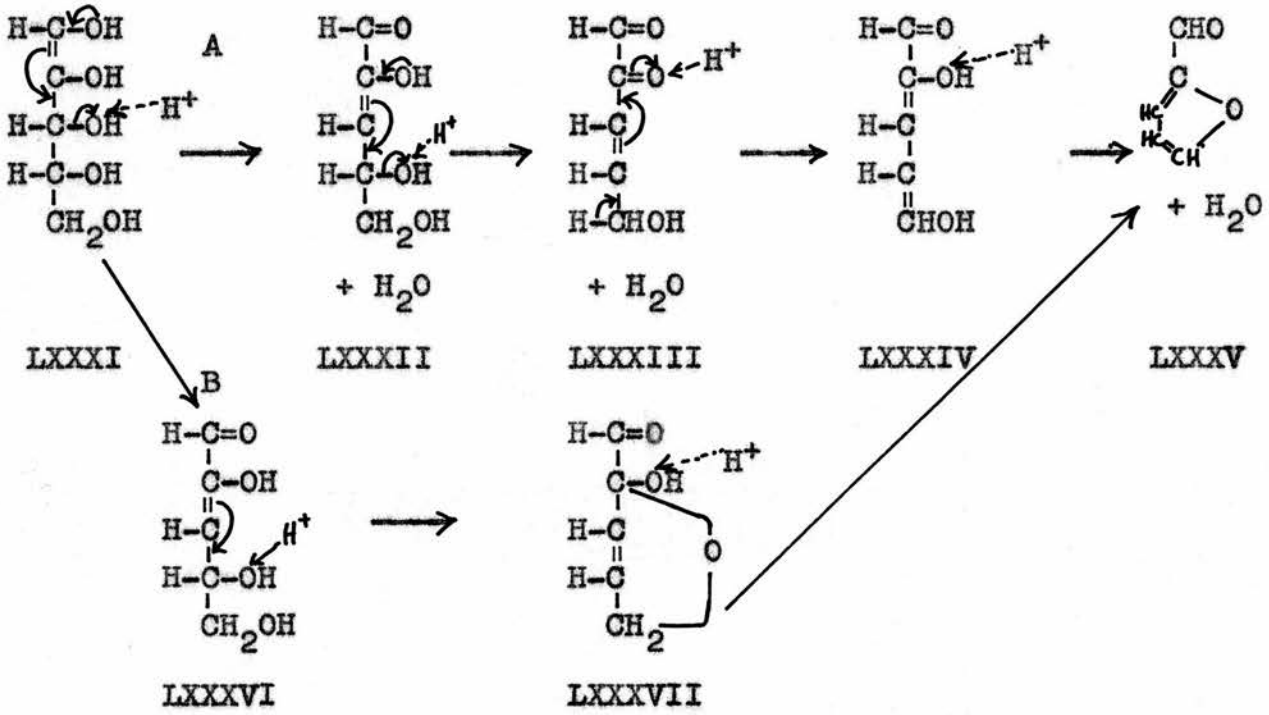


LXXIX



LXXX

The formation of furfural from pentoses has been formulated as follows (69) (161)



In path A the first water molecule is eliminated in the position B to the carbonyl group. The second is then eliminated in the position adjacent to the double bond. The unsaturated osone, LXXXIII, rearranges to the enol form. This dehydrates with ring formation to give furfural, LXXXV. The scheme B is similar to this, but ring closure is followed by water elimination in the last step. None of these intermediates have been isolated.

Similar mechanisms have been put forward for the formation of hydroxy methyl furfural from aldo- and keto-hexoses and for methyl furfural formation from methyl pentoses.

The rate of furfural formation and yield have been investigated

by Love (174) for various sugars in 28N.H_2SO_4 at 60°C. The time required to reach the maximum intensity of absorption at 315 mμ (for furfural) and 320 mμ (for hydroxymethyl furfural) was noted along with the maximum extinction. He found that enantiomorphic sugars degrade to the same extent at the same rate. D-xylose gave furfural faster and to a greater extent than its C-4 epimer L-arabinose. D-glucose with a pyranose ring similar to D-xylose forms hydroxymethyl furfural slower but to a greater extent than D-galactose, which has a similar pyranose ring to L-arabinose. Fructose gives the highest yield of 5-hydroxymethyl furfural (5-HMF); under similar conditions sorbitol, mannitol and gluconic acid give no U.V. absorption, implying no decomposition under these conditions.

2,3,4,6-O-tetramethyl-D-glucopyranose is more stable in acid than D-glucose (175). In boiling 8% HCl it gives practically no 5-HMF; in contrast, 1,3,4,6-O-tetramethyl-D-fructofuranose gives as good a yield of 5-HMF as D-fructose under these conditions.

Apart from furfural compounds, the attack of acid on hexoses and pentoses yields formaldehyde, acetaldehyde, and crotonaldehyde (176) (177). The ease of formation of aldehydic compounds from pentoses and hexoses was estimated.

In 4N.H_2SO_4 at room temperature the following order was found.

Gulose > talose > galactose > altrose > mannose > glucose.

In 20N.H_2SO_4 at room temperature

Talose > mannose > galactose > glucose > altrose

and for pentoses the following order:-

In 4N.H_2SO_4

Lyxose > ribose > xylose > arabinose.

In 20N.H_2SO_4

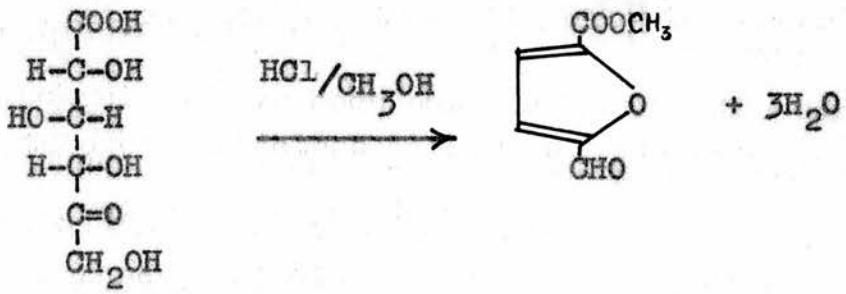
Xylose > ribose > lyxose > arabinose.

Many colorimetric methods of sugar determination depend on the action of heated acid on the sugars (See Part VI). Acid hydrolysis is also commonly used to elucidate the structure of polysaccharides.

2) The dehydration of alduronic and keturonic acids.

In general, alduronic and keturonic acids are dehydrated and decarboxylated in acidic media more easily than the related polyhydroxy mono- and di-carboxylic acids. Mechanisms similar to those for the polyhydroxy carbonyl compounds have been postulated; the reactions were mainly followed by CO_2 liberation and analysis of the end-product.

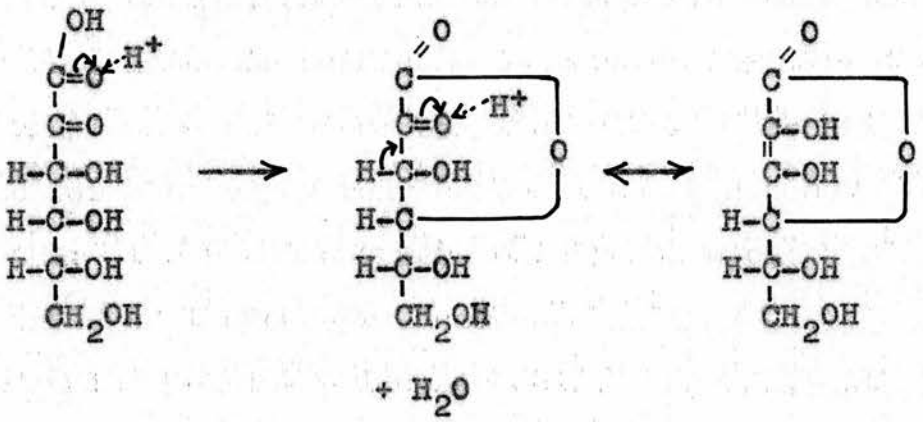
Refluxing 5-keto-D-gluconic acid, LXXXVIII, with 30% HCl in CH_3OH gives the ester of 5-formyl furoic acid, LXXXIX (178), which gives the acid itself on hydrolysis.



LXXXVIII LXXXIX

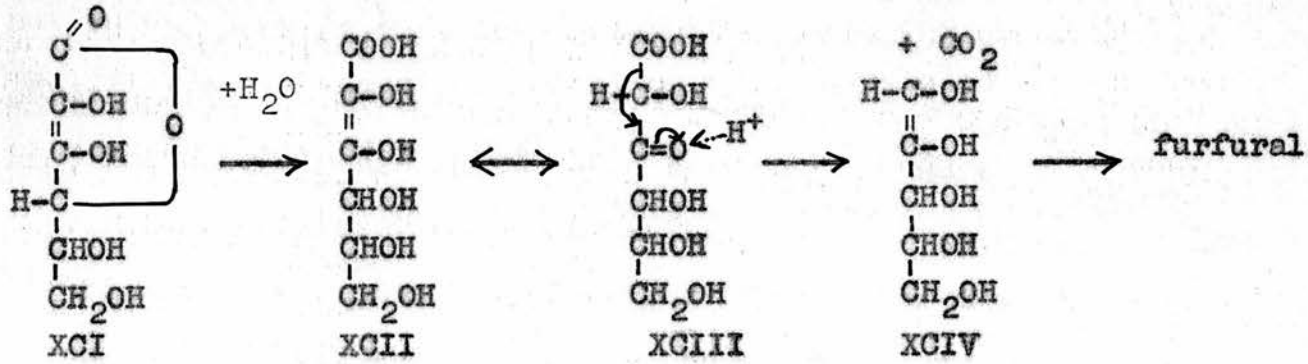
In 12% HCl, 5-keto-L-galactonic acid decarboxylates to furfural (179), a yield of 42% of theoretical being similar to that from D-galacturonic acid but the rate of formation is much greater.

2-Keto-hexaldonic acids are easily converted to furfural in boiling 12% HCl, the yield being 30% after 4 hrs. It is possible that ascorbic acids are the first intermediates (99) (180). Ascorbic acids are stable lactone diols, the double bond being stabilised by conjugation with the carbonyl double bond. Lactonisation and enediolisation converts a 2-keto-hexaldonic acid, XC, to an ascorbic acid, XCI, by the following reaction (69)



XC XCI

Further dehydration to pentose and hence to furfural has been suggested (179)



XCI XCII XCIII XCIV

The lactone ring opens, the enediol can then give the β-keto acid which decarboxylates easily to the pentose, XCIV. According to this the formation of furfural from ascorbic acid goes by the intermediate pentose enediol.

Regna and Caldwell (102) measured the rate of formation, in 5N.HCl, of ascorbic acids from 2-keto hexaldonic acid and also of furfural from the ascorbic acids. (The results are shown in Tables VII(m) and VII(n), pp. 72, 73). The activation energies for similar reactions are approximately equal within the experimental error, showing the same mechanism to be operative in each case. Table XVI compares the yield of ascorbic acid as calculated from the rate equation with the values found experimentally.

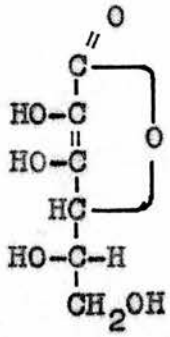
TABLE XVI

The yield of ascorbic acid from 2-keto-hydroxy acids at 59.9°C and 69.9°C in 5N.HCl (102).
Yields calculated from k_1 and k_2 of Tables VII(m) and (n).

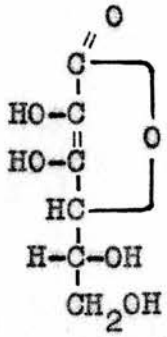
2-keto hexonic acid	Temp.	Maximum yield of ascorbic acid as % of theoretical	
		Calculated	Found
L-gulonic	59.9	67.5	67.6
	69.9	69.4	69.9
D-gluconic	59.9	20.5	20.8
	69.9	24.3	24.4
D-galactonic	59.9	42.1	41.7
	69.9	44.3	52.5
D-glucoheptonic	59.9	25.4	27.7
	69.9	27.6	28.2
D-galactoheptonic	59.9	6.3	6.1
	69.9	5.2	7.3

The yield of ascorbic acid depends on the position of all the OH groups. 2-Keto-L-gulonic acid should give the same yield as 2-keto-D-gluconic acid, whereas it gives much more.

Tables VII(m) and (n) show that L-xyloascorbic acid, XCV, is more quickly formed from 2-keto-L-gulonic acid than is D-araboascorbic acid, XCVI, from 2-keto-D-gluconic acid. However, the D-araboascorbic acid is more quickly converted to furfural than is D-xyloascorbic acid.



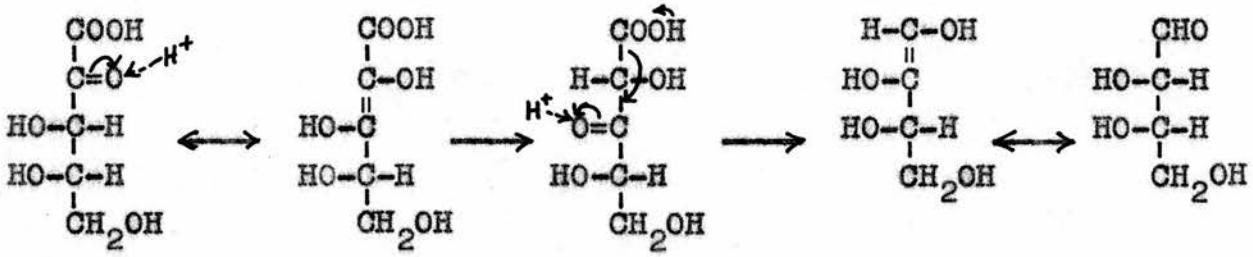
XCV



XCVI

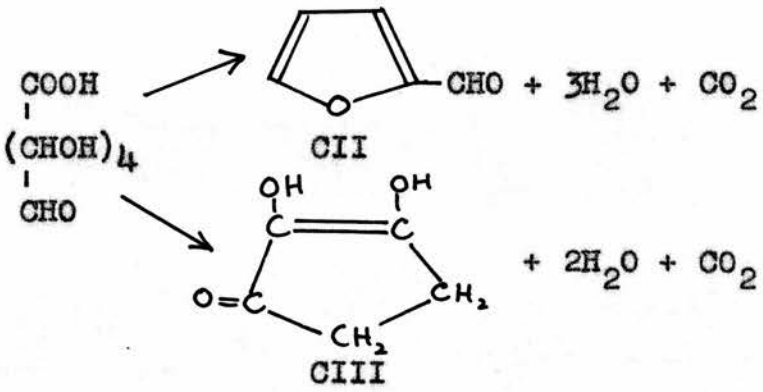
The position of the OH-group on the C-5 atom is seen to be significant although the mechanisms put forward for the reaction do not indicate the nature of this effect. It is also seen from Table XVI that there is a difference between the C-6 epimeric heptonic acids.

2-Keto-L-arabonic acid, XCVII, is decomposed in 8% H_2SO_4 to L-erythrose (181). The intermediate, C, is finally formed by enediolisation to XCVIII and ketonisation to XCIX followed by decarboxylation. Further dehydration and ring closure is not possible so that the tautomeric L-erythrose, CI, is stable.



XCVII XCVIII XCIX C CI

In addition to furfural, CII, the acid treatment of hexuronic acids gives rise to reductic acid, CIII.



CII CIII

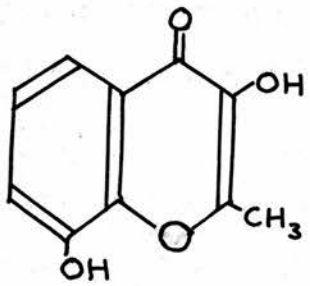
Since reductic acid has two hydroxyl groups it can be assumed that it is formed in greater amounts under less vigorous dehydration conditions; this is found to be so. Table IV (p.43) shows the results obtained by Stutz and Deuel (65), the amount of reductic acid formed increases as the amount of furfural formation decreases.

Penturonic acids give only approximately 50% decarboxylation (71) and no dehydration intermediates were isolated.

Threuronic acid, a tetrauronic acid, dehydrates in acid solution to a β-keto acid, giving methyl glyoxal on decarboxylation (95).

Glyoxylic acid, a diuronic acid, is stable in hot 12% HCl (83).

As well as furfural and reductic acid, 2-methyl-3,8-dihydroxy-chromone, CXIII, has been isolated (208).



CXIII

3) Dehydration of mono- and dibasic polyhydroxy carboxylic acids.

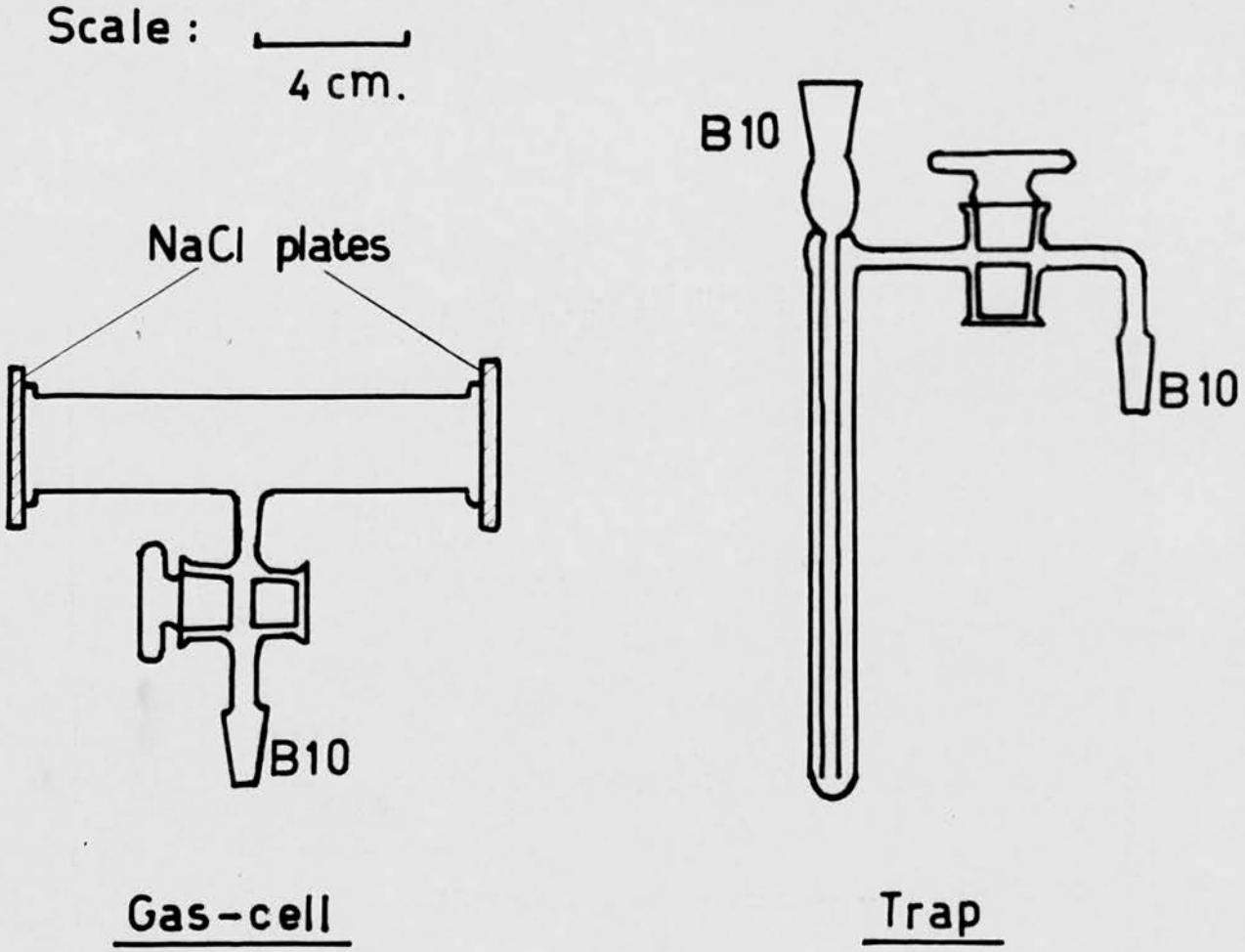
Monobasic polyhydroxy carboxylic acids and their lactones dehydrate much more slowly than the similar uronic acids.

In 28N.H_2SO_4 at 60°C, gluconic acid, with long reaction time, gives no selective absorption in the U.-V. (174).

Galactonic acid, and talonic acid (209), give a poor yield of 5-hydroxymethyl-2-furan carboxylic acid. Rhamnolactone is dehydrated to 3-hydroxy-6-methylpyrone, , (210). Arabonic acid gives a good yield of 2-furan carboxylic acid (211).

Saccharic acids, e.g. glucaric acid, dehydrate on heating in mineral acid to give 2,5-furan dicarboxylic acid (212). Mucic acid gives a good yield of this compound when heated in concentrated HBr. (213), CO_2 only being liberated (70). As well as 2,5-furan dicarboxylic acid in 42% yield, benzofuran is formed in 12% yield.

These then are the products which have been reported for the acid decomposition of carbohydrate materials. It was therefore decided to analyse all the gaseous products formed by the attack of boiling 19% HCl on many carbohydrate and naturally occurring compounds.



Gas-cell

Trap

FIG. 8

EXPERIMENTAL

The apparatus used was a modification of that shown in Fig. 1. The CO_2 absorption trap was replaced by one of special design (182) in which the gaseous products were trapped out in liquid oxygen. The products were then transferred (182) to a gas-cell and analysed by infra-red spectroscopy. The trap and cell are shown in Fig. 8.

Weighed samples of the materials to be investigated were placed in the reaction flask, 50 ml. 19% (w/w) HCl were added, and the mixture boiled for the time indicated. Nitrogen was used as scrubbing gas, the vapour being first passed through an 'anhydrone' tube to remove traces of water before trapping out in liquid oxygen.

THE UNIVERSITY *of* EDINBURGH

PAGE MISSING IN ORIGINAL

EXPERIMENTAL RESULTS.

The products from the decomposition of the materials in 19% HCl were characterised by a direct comparison with spectra of known compounds. These standard spectra were obtained from commercially available reagents. The following standard spectra were obtained:- Acetaldehyde, acetic acid, acetone, acrolein, allyl acetate, chloral, crotonaldehyde, ethyl acetate, ethyl chloride, ethyl chloroacetate, ethyl chloroformate, ethyl orthoformate, ethylene dichloride, ethyl formate, formaldehyde, formic acid, furan, 2,5-dimethyl furan, 2-methyl furan, furfural, methyl acetate, methyl acrolein, methyl alcohol, methyl chloride, methyl chloroformate, methyl formate, methyl ethyl ketone, diethyl ketone, propionaldehyde.

In some cases decomposition products other than those listed above were obtained and hence it was not found possible to characterise them. These are listed in Table XVII as unidentified compounds.

Table XVII shows the products obtained for various reaction times; similar compounds are grouped together.

TABLE XVII

The gaseous decomposition products obtained on boiling various carbohydrates and related compounds in 19% (w/w) HCl for various times.

tr. = trace. ++ = large amount. + = medium amount.

Compound	Time (hrs.)	Soln. Colour	Products				
			CO_2	Furan	CH_3CHO	CH_3COCH_3	Furfural
D-glucurone (200 mg.)	0-3	Dark brown	++	++	-	-	-
	3-9		++	++	tr.	-	-
	9-12		++	tr.	++	-	-
	12-24		+	tr.	+	+	-
	24-48		+	-	tr.	tr.	+
	48-72		+	-	-	-	-
D-galacturonic acid monohydrate (250 mg.)	0-6	Dark brown	++	++	-	-	-
	6-9		++	++	tr.	-	-
	9-12		++	+	+	+	-
	12-24		+	tr.	+	+	-
	24-48		+	-	-	-	+
	48-72		+	-	-	-	-
5-Keto-D-gluconate-Ca-salt (280 mg.)	0-7	Dark brown	++	++	-	-	-
	7-12		++	++	tr.	-	-
	12-24		++	+	tr.	tr.	-
	24-48		+	-	-	+	tr.
	48-72		tr.	-	-	tr.	tr.
D-xylose (1 g.)	0-12	Dark brown	+	++	-	-	-
	12-24		+	++	tr.	-	-
	24-48		+	-	tr.	tr.	+
	48-72		+	-	-	-	+
L-arabinose (1 g.)	0-12	Dark brown	+	++	-	-	-
	12-24		+	++	+	-	-
	24-48		+	-	tr.	-	+

TABLE XVII (Continued)

Compound	Time (hrs.)	Soln. Colour	Products				
			CO_2	Furan	CH_3CHO	CH_3COCH_3	Furfural
Furfural (500 mg.)	0-24	Dark brown	+	++	-	-	-
	24-48		+	tr.	-	-	tr.
	48-72		+	-	-	-	-
5-Hydroxymethyl furfural (500 mg.)	0-24	Dark brown	+	tr.	-	-	-
	24-48		+	-	-	tr.	-
	48-72		+	-	-	tr.	-
D-glucose (500 mg.)	0-48	Dark brown	+	-	-	-	-
D-galactose (500 mg.)	0-4	Dark brown	+	+	-	-	-
	4-24		+	+	tr.	tr.	-
	24-48		+	-	tr.	tr.	-
D-glucoheptose (180 mg.)	0-24	Dark brown	+	-	-	-	-
	24-48		+	-	-	tr.	-
D-mannose (500 mg.)	0-24	Dark brown	+	tr.	-	-	-
	24-48		+	-	tr.	-	-
Chloralose (500 mg.)	0-24	Dark brown	+	tr.	-	-	-
	24-48		+	-	tr.	-	-
L-sorbose (500 mg.)	0-24	Dark brown	+	tr.	-	-	-
	24-48		+	tr.	tr.	tr.	-
D-fructose (500 mg.)	0-24	Dark brown	+	-	-	-	-
	24-48		+	-	-	-	-
D-lyxose (500 mg.)	0-24	Dark brown	+	+	tr.	-	-
	24-48		+	+	tr.	tr.	-
D-ribose (180 mg.)	0-24	Dark brown	+	+	+	+	-
	24-48		+	-	-	tr.	+
D-erythrose (180 mg.)	0-24	Dark brown	+	-	-	-	-
	24-48		+	-	tr.	-	-

TABLE XVII (Continued)

Compound	Time (hrs.)	Soln. Colour	Products				
			CO_2	Furan	CH_3CHO	CH_3COCH_3	Furfural
DL-glyceraldehyde (350 mg.)	0-24	Pale brown	tr.	-	tr.	-	-
	24-48		tr.	-	tr.	tr.	-
meso-inositol (500 mg.)	0-48	Colourless	tr.	-	-	-	-
Mannitol (500 mg.)	0-24	Colourless	tr.	-	tr.	tr.	-
	24-48		tr.	-	-	-	-
Sorbitol (500 mg.)	0-24	Colourless	tr.	-	-	tr.	tr.
	24-48		tr.	-	-	-	-
Dulcitol (500 mg.)	0-48	Colourless	tr.	tr.	-	tr.	-
Ribitol (180 mg.)	0-48	Colourless	tr.	tr.	-	tr.	-
Digitoxose (180 mg.)	0-24	Dark brown	+	-	+	-	-
	24-48		+	-	+	tr.	-
Sucrose (500 mg.)	0-24	Dark brown	+	tr.	-	-	-
	24-48		+	tr.	tr.	tr.	-
Cellobiose (500 mg.)	0-24	Dark brown	+	tr.	-	-	-
	24-48		+	-	-	tr.	-
Melibiose (500 mg.)	0-24	Dark brown	+	tr.	-	-	-
	24-48		+	-	-	tr.	-
Lactose (500 mg.)	0-24	Dark brown	+	tr.	-	-	-
	24-48		+	-	-	tr.	-
Maltose (500 mg.)	0-24	Dark brown	+	tr.	-	-	-
	24-48		+	-	-	tr.	-
Trehalose (500 mg.)	0-24	Dark brown	+	-	-	-	-
	24-48		+	-	-	-	-
Melezitose (500 mg.)	0-24	Dark brown	+	tr.	-	-	-
	24-48		+	-	-	-	-

TABLE XVII (Continued)

Compound	Time (hrs.)	Soln. Colour	Products				
			CO_2	Furan	CH_3CHO	CH_3COCH_3	Furfural
D-galactonic acid-γ-lactone (500 mg.)	0-24	Very pale yellow	+	+	-	-	-
	24-48		+	+	-	+	-
Gluconic acid-δ-lactone (500 mg.)	0-24	Pale brown	+	tr.	-	-	-
	24-48		+	tr.	-	-	-
Gulonic acid (500 mg.)	0-24	Pale brown	+	+	-	-	-
	24-48		+	+	-	-	-
Ca-L-idonate (250 mg.)	0-24	Pale brown	+	+	-	-	-
	24-48		+	-	-	-	-
D-ribonic acid-γ-lactone (500 mg.)	0-24	Pale brown	+	+	-	-	-
	24-48		+	+	-	-	-
D-arabonic acid-γ-lactone (500 mg.)	0-24	Colour-less	+	+	-	-	-
	24-48		+	+	-	-	-
2-Keto-L-galactonic acid (128 mg.)	0-24	Dark brown	++	++	+	-	-
	24-48		+	+	+	-	-
L-ascorbic acid (180 mg.)	0-24	Dark brown	++	++	+	-	-
	24-48		+	tr.	+	+	+
iso-ascorbic acid (180 mg.)	0-3	Dark brown	++	+	-	-	-
	3-24		+	+	+	-	-
	24-48		+	-	-	+	+
Mucic acid (500 mg.)	0-24	Pale brown	+	+	-	-	-
	24-48		+	+	-	-	-
L-gulonic acid-γ-lactone (270 mg.)	0-24	Colour-less	+	tr.	-	-	-
	24-48		+	tr.	-	-	-

TABLE XVII (Continued)

Compound	Time (hrs.)	Soln. Colour	Products				
			CO_2	Furan	CH_3CHO	CH_3COCH_3	Furfural
Glucosamine hydrochloride (500 mg.)	0-24	Dark brown	+	+	-	-	-
	24-48		+	+	-	-	-
N-methyl-1-D-glucamine (500 mg.)	0-24	Colourless	tr.	tr.	-	tr.	-
	24-48		tr.	-	-	-	-
Glucosaminic acid (300 mg.)	0-24	Dark brown	++	+	-	-	-
	24-48		+	tr.	-	-	-
Furoic acid (176 mg.)	0-24	Pale brown	++	+	-	-	-
	24-48		+	+	-	-	-
5-Carboxy-furoic acid (500 mg.)	0-24	Colourless	+	+	-	-	-
	24-48		+	+	-	-	-
Reductic acid (131 mg.)	0-24	Pale brown	+	-	-	-	-
	24-48		+	-	-	-	-
Meconic acid (111 mg.)	0-24	Dark brown	++	++	-	-	-
	24-48		+	+	-	-	-
Kojic acid (150 mg.)	0-24	Dark brown	+	-	+	-	-
	24-48		+	-	+	-	-
Muconic acid (250 mg.)	0-24	Colourless	++	-	-	tr.	-
	24-48		+	-	-	tr.	-
Tartronic acid (500 mg.)	0-24	Colourless	++	-	-	-	-
	24-48		+	-	-	tr.	-
α-Keto-glutaric acid (500 mg.)	0-24	Dark brown	+	-	-	-	-
	24-48		+	-	tr.	tr.	-
2-Keto-gulonic acid (200 mg.)	0-24	Dark brown	++	tr.	-	+	-
	24-48		+	-	-	+	tr.

TABLE XVII (Continued)

Compound	Time (hrs.)	Soln. Colour	Products				
			CO_2	Furan	CH_3CHO	CH_3COCH_3	Furfural
Dihydroxy acetone (350 mg.)	0-24	Brown	+	-	tr.	-	-
	24-48		+	-	+	+	-
Diglycollic acid (500 mg.)	0-24	Brown	+	-	-	tr.	-
	24-48		+	-	-	tr.	-
Glycolaldehyde (450 mg.)	0-24	Dark brown	+	-	+	-	-
	24-48		+	-	-	tr.	-
β-keto-adipic acid (300 mg.)	0-24	Colour-less	+	-	-	-	-
	24-48		+	-	-	-	-
Aconitic acid (400 mg.)	0-24	Colour-less	+	-	-	-	-
	24-48		+	-	-	tr.	-
Itaconic acid (500 mg.)	0-24	Colour-less	tr.	-	-	-	-
	24-48		tr.	-	-	-	-
Na-glyoxylic acid (350 mg.)	0-24	Pale brown	+	-	-	-	-
	24-48		+	-	-	-	-
Allantoin (500 mg.)	0-24	Colour-less	++	-	-	-	-
	24-48		+	-	-	-	-
Alloxantin (500 mg.)	0-24	Colour-less	++	-	-	-	-
	24-48		+				
Hydantoin (134 mg.)	0-48	Colour-less	++	-	-	-	-
Hydantoic acid (109 mg.)	0-48	Colour-less	++	-	-	-	-
Hypoxanthine (126 mg.)	0-48	Bright yellow	++	-	-	-	-
Orotic acid (208 mg.)	0-48	Colour-less	++	-	-	-	-
Xanthine (295 mg.)	0-48	Colour-less	++	-	-	-	-

TABLE XVII (Continued)

Compound	Time (hrs.)	Soln. Colour	Products				
			CO_2	Furan	CH_3COCH_3	CH_3CHO	Other products
Lævulinic acid (500 mg.)	0-24	Colourless	tr.	-	-	-	
	24-48		tr.	-	-	-	tr. chloral; tr. furfural
Pyruvic aldehyde (500 mg.)	0-24	Dark brown	+	-	-	+	Propionaldehyde
	24-48		+	-	-	+	Propionaldehyde
Chelidonic acid (115 mg.)	0-24	Pale brown	++	-	tr.	-	
	24-48		+	-	tr.	-	tr. chloral
Reductone (500 mg.)	0-24	Black-brown	+	-	-	tr.	tr. chloral
	24-48		+	-	-	tr.	tr. chloral
α-D-gluco-heptono-γ-lactone (188 mg.)	0-24	Dark brown	++	-	-	-	tr. chloral
	24-48		+	tr.	tr.	-	tr. chloral
Ca-gluco-heptonate (250 mg.)	0-24	Dark brown	++	+	tr.	-	tr. chloral; tr. 2-Me-furan
	24-48		+	tr.	tr.	tr.	tr. chloral
D-galaheptono-γ-lactone (133 mg.)	0-24	Dark brown	++	tr.	-	-	tr. chloral
	24-48		+	tr.	+	-	chloral
Erythritol (500 mg.)	0-24	Colourless	+	tr.	tr.	-	Unknown keto compound; ++
	24-48		+	tr.	tr.	-	
2-deoxy-D-glucose (250 mg.)	0-24	Dark brown	+	-	tr.	-	tr. diethyl ketone
	24-48		+	-	tr.	-	tr. diethyl ketone
2-deoxy-D-galactose (275 mg.)	0-24	Dark brown	+	-	-	-	tr. diethyl ketone
	24-48		+	-	-	-	tr. diethyl ketone

A large amount of an unknown ketone is formed from erythritol. It has much the same spectrum as methyl ethyl ketone with a slight hydroxyl and a much modified CH peak at 3018 cm^{-1}. The mode of formation of chloral is not clear.

TABLE XVII (Continued)

Compound	Time (hrs.)	Soln. Colour	Products				Other products
			CO_2	Furan	CH_3CHO	CH_3COCH_3	
5-Methyl furfural (500 mg.)	0-1	Colourless	+	tr.	-	-	2-Me.-furan.
	1-24		+	+	-	-	2-Me.-furan; Me.-formate.
	24-48		+	-	-	+	Furfural; Me.-formate.
	48-72		+	-	-	-	-
L-fucose (500 mg.)	0-24	Dark brown	+	tr.	-	-	2-Me.-furan.
	24-48		+	-	-	tr.	tr. 2-Me.-furan.
L-rhamnose (500 mg.)	0-24	Dark brown	+	-	tr.	-	2-Me.-furan.
	24-48		+	-	+	-	
Maltol (500 mg.)	0-24	Dark brown	+	-	tr.	-	2-Me.-furan.
	24-48		+	-	+	-	tr. 2-Me.-furan.
Ethylene glycol (500 mg.)	0-24	Colourless	-	-	+	-	+ 1,2-dichloroethane.
	24-48		-	-	tr.	-	tr. 1,2-dichloroethane.
Coumalic acid (360 mg.)	0-24	Pale brown	++	-	-	-	++ Crotonaldehyde
	24-48		+	-	+	-	+ Crotonaldehyde

Although the well-known products from pentoses, etc., are furfural derivatives, some of these are too involatile to come over initially under the experimental conditions. It can be seen that all the compounds tested gave some CO_2, but the furfural compounds must decompose to a certain extent, giving rise to the appropriate furan derivative, presumably by decarbonylation. Only after 24 hrs. does furfural appear in small amounts. Acetaldehyde and acetone are

presumably eliminated during the formation of the dark polymeric residues.

The unknown aldehydic and keto-compounds have quite simple spectra and may be chloro-derivatives of simple aldehydes and ketones; spectra of these were, however, not available.

2-Methyl furan and methyl formate will not interfere in the quantitative estimation of uronic acids. With methylated and esterified sugars the volatile products include methyl chloride, which will interfere with the estimation by the decarboxylation method; hence with these compounds present large errors could occur.

With and without metal acetates in boiling water, galacturonic acid gave only CO_2 as the volatile product.

With 19% HCl and using oxygen or hydrogen in place of nitrogen as flow-gas, no change in products was found.

In the case of glucose (B.D.H. microanalytical reagent), it is seen that a trace of CO_2 is the only product of the decomposition. It is possible, then, that in the case of the other hexoses, the gaseous products other than CO_2 are due to impurities. This may also be true for the aldonic acids, some of which give clear colourless solutions with CO_2 and furan as the only products, while others give pale brown solutions with various products.

Further work on purified samples should be carried out using this method to determine the impurities present, if any. The

above samples were all commercial samples and as such are unlikely to be absolutely pure. This, then, provides a method of testing the purity of a given sample of hexose, aldonic acid or hexitol, the main impurity being pentose, which gives rise to furan and various carbonyl compounds.

PART V

The thermal decarboxylation of some uronic acids and the high temperature liberation of CO_2 from non-uronic acid materials.

INTRODUCTION

The first investigation of the thermal decomposition of carbohydrate materials was carried out by Puddington (159) who measured the rate of reaction and composition of the products formed at 10^{-5}m.m. over a temperature range of 150-240°C at times ranging from 15 min. to 8 days. The main volatile products found were water, CO_2, and CO; small quantities of acids, aldehydes and other products were also determined. Cellobiose was found to decompose in two reaction stages - both substantially first order - with a change in activation energy from over 60 kcals. to 40 kcals. after a loss of 10% water; the molar ratio of water : CO_2 : CO was 64 : 4 : 1, at high temperatures. However, maltose appeared to give a single stage reaction with an activation energy of 35 kcals., calculated for a first order reaction.

Perlin (66) found that carbon dioxide was also liberated from uronic acids by merely heating the material without the use of solvent or added catalyst. He found that the decarboxylation of uronic and polyuronic acids and salts was nearly quantitative in 15 mins. at 255°C. The uronic acid materials are strongly dehydrated and the residue obtained was analysed, the analysis corresponding to a 5-carbon skeleton containing 1.5 atoms of oxygen. Other minor products of the reaction were found to be carbon monoxide and traces of acids and "oils". Sugar acids, such as gluconic and ascorbic, were not so extensively decarboxylated. In the analysis for uronic acid content of various oxidised

starches, good agreement with the titration and 12% HCl decarboxylation method was obtained. The apparatus was not fully described, but appears to have been quite a simple tube with an inlet for the scrubbing-gas (CO_2-free nitrogen), which passed over the heated sample, so removing any CO_2 formed. This gas was then passed through weighed ascarite tubes in which the CO_2 was absorbed.

Since this was reported, by Perlin, to be a good method for the estimation of uronic acids, it was decided to attempt to repeat and expand the above work with a view to improving, if possible, the technique.

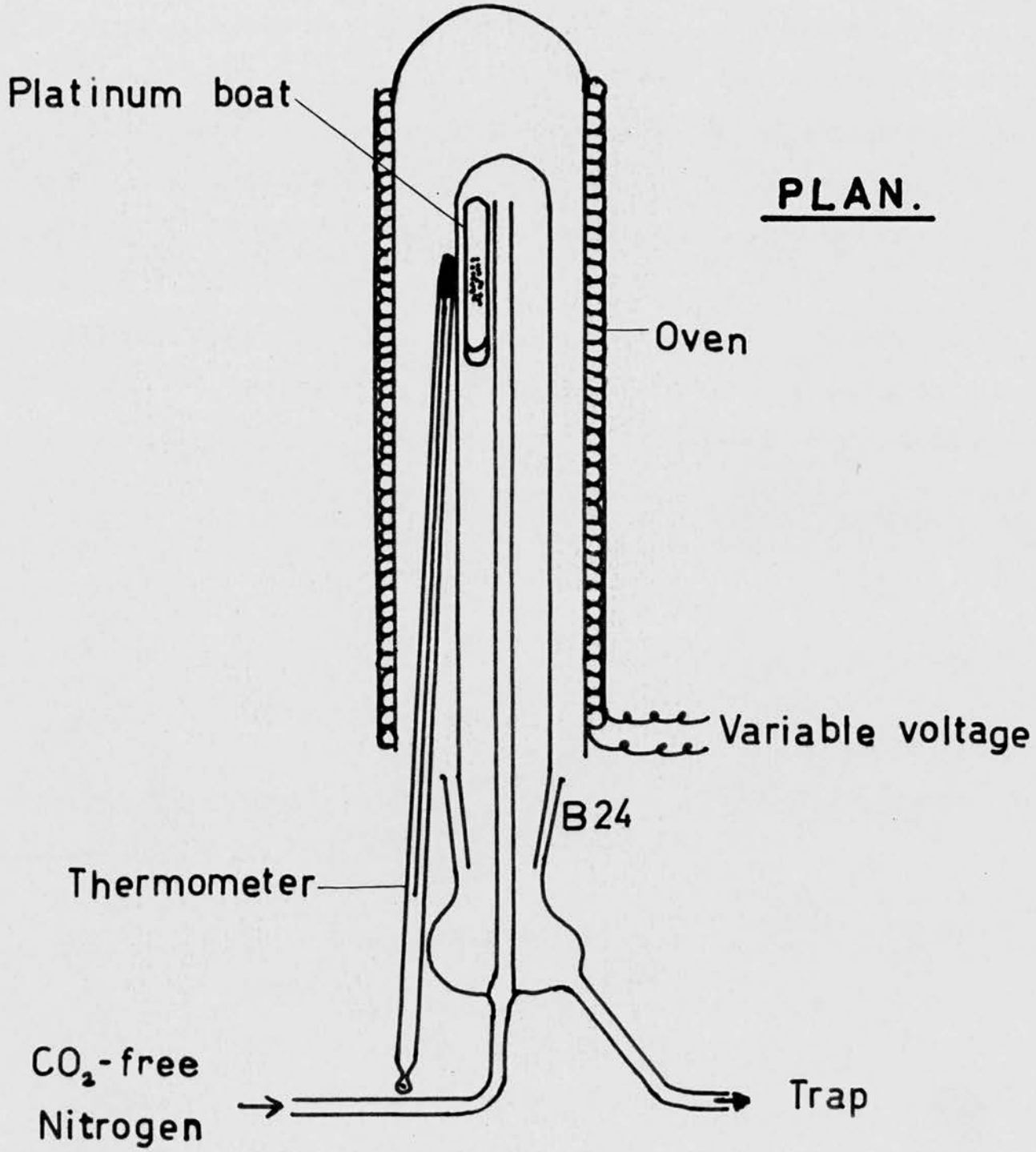

Platinum boat
PLAN.
Oven
Variable voltage
B24
Thermometer
CO_2-free
Nitrogen
Trap

FIG. 9

EXPERIMENTAL

The apparatus is shown diagrammatically in Fig. 9. Analytical grade nitrogen, at a flow-rate of 15 ml./min., is passed first through "Sofnolite" to remove any carbon dioxide and then through "Anhydrone" to remove moisture. This gas then passes through the apparatus scrubbing out the gas inside the oven and passing into an absorption trap of the same design as shown in Fig. 1; in kinetic runs two matching traps were used, the CO_2 being absorbed in standard baryta. For the analysis of the other gaseous products from the decomposition, the absorption traps were replaced by the traps shown in Fig. 8, in which the products are condensed out in liquid oxygen and analysed by infra-red spectroscopy in the gaseous state.

Since Perlin had shown that the decarboxylation was virtually independent of pressure, determinations were all carried out at atmospheric pressure.

Approximately 30 mg. of material were accurately weighed out in a platinum boat, the boat being cleaned with 1 : 1, HNO_3 : water and ignited before use. The boat was then placed in the combustion tube so that the outflowing nitrogen passed over the material. The oven already heated to the required temperature was then placed round the tube and zero time taken as 7 min. after this time to allow for the delay in sweeping over from the tube. The temperature of the oven round the combustion tube could be varied by means of a "Variac" variable voltage transformer. The excess baryta was back titrated at the end of each run and the amount of

CO_2 estimated as in the acid decarboxylation experiments.

The galacturonic acid monohydrate, glucurone and alginic acid were the same samples as were used for the acid decarboxylation reaction.

EXPERIMENTAL RESULTS

The purity of the galacturonic acid monohydrate, glucurone and alginic acid was taken to be the same as found by titration (see p.48).

30 mg. samples of the above uronic acids were heated at various temperatures and the amount of CO_2 given off estimated at various times. The results are shown in Tables XVIII(a), (b) and (c).

TABLE XVIII(a)

The thermal decarboxylation of galacturonic acid monohydrate.

Results given as % moles CO_2 per mole.

Time (hrs.)	Temp.		
	220°C	250°C	300°C
1	40.7	71.0	105.0
2	47.5	85.6	115.1
4	57.0	99.1	128.8
10	71.2	118.3	148.2
20	86.6	139.8	174.0

The tables show that the amount of carbon dioxide liberated increases with temperature, the value being very dependent on the temperature used. It seems doubtful from this and the following tables whether this could ever be as satisfactory a method of uronic acid estimation as was suggested by Perlin.

TABLE XVIII(b)

The thermal decarboxylation of glucurone.

Results given as % moles CO_2 per mole.

Time (hrs.)	Temp.		
	220°C	250°C	300°C
1	21.8	70.6	91.3
2	32.3	82.4	98.2
4	46.2	91.6	104.1
10	65.0	104.5	117.0
20	75.4	111.8	131.3

TABLE XVIII(c)

The thermal decarboxylation of alginic acid.

Results given as % moles CO_2 per mole.

Time (hrs.)	Temp.		
	220°C	250°C	300°C
1	40.9	71.5	84.1
2	47.1	79.8	94.2
4	55.0	88.1	109.1
10	65.2	103.2	134.2
20	75.4	120.8	157.1

The values at the higher temperatures were somewhat variable, the above being the mean of a number of runs.

It can be seen that at 300°C the titrimetric value is reached between 2 and 4 hrs. for glucurone and alginic acid, but

galacturonic acid decomposes much more easily, giving the analytical value in approx. 45 mins.; none give the theoretical in 15 mins. at these temperatures, as was claimed by Perlin.

It is clearly seen that much of the uronic acid molecule is decomposing as well as the carboxyl group.

As reported by Puddington, sugars also give CO_2; the results for arabinose, xylose and glucose are given in Table XVIII(d).

TABLE XVIII(d)

The liberation of CO_2 from non-uronic acid carbohydrates at 300°C (50 mg. samples taken).

Results as % moles CO_2 per mole.

Time (hrs.)	D-glucose	L-arabinose	D-xylose
2	16.5	13.8	11.3
4	20.4	17.6	16.2
6	24.6	20.3	20.0
8	27.9	22.2	25.7
20	43.5	36.4	44.1

It is seen that large amounts of CO_2 are liberated by these sugars, indicating a breakdown of the actual molecule. Evidently the carboxyl group of the uronic acid is not all freed in some cases but may be incorporated in the polymer formed. The other gaseous products from this reaction were also analysed by infra-red spectroscopy. From the uronic acids, only CO_2 was detected in the trapped gases; with the other sugars, CO_2 and also a trace of acetaldehyde was found.

PART VI

The colorimetric estimation of uronic acid content of carbohydrate materials.

INTRODUCTION

Although the most generally applicable method of uronic acid estimation is by decarboxylation, a large number of colorimetric methods are known which depend on the reaction of a reagent with either the uronic acid or, more generally, with its decomposition products.

A possible colorimetric reaction is:-

$$RCOOH + M^{+} \rightleftharpoons \underset{\text{(coloured)}}{RCOOM} + H^{+}$$

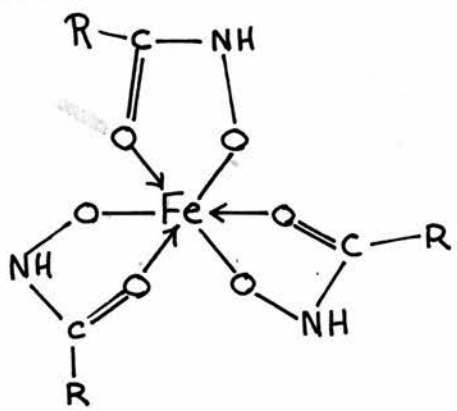
Since this reaction is an equilibrium, conditions have to be found which will give quantitative reaction. In this case the amount of cation M^{+} absorbed from solution is a stoichiometric measure of the carboxyl content of the uronic acid.

The absorption of Methylene blue from solution is an example of this type of reaction and has been thoroughly investigated by Davidson (183), who found errors in the carboxyl values at low carboxyl content, due to absorption by -OH groups.

It is well known that the reaction between an ester and hydroxylamine gives a hydroxamic acid (184) which can then react with ferric ions to give stable ferric complexes.

$$RCOOR + NH_2OH = R\text{-}CONHOH + R\,OH$$

$$3\ R\text{-}CO\text{-}NHOH + Fe^{+++} =$$



Kaye and Kent (185) applied this reaction under carefully controlled conditions to the determination of uronic acids in polysaccharides. They found that the reaction appeared to go as well with internal esters (such as lactones) as with ordinary esters and so used glucurone as standard to estimate the uronic acid content of various polysaccharides. The values obtained for glucurone in the lactone form and in the methyl ester form were in agreement when the lactone absorption had been corrected for the absorption due to the aldehydic group by subtraction of the value obtained for glucose from the glucurone curve.

Hilf and Castano (186), evidently unaware of the above work, also used the hydroxamic acid formation as a means of estimating reducing sugars by first converting the sugar to the cyanohydrin derivative which gives the acid (or lactone under acid conditions) and hence the hydroxamic acid derivative which then reacts with Fe^{+++} to give the typical colour reaction. The method appears to be rather tedious but the authors claim quantitative recovery.

Lien (187) also applied the iron-hydroxamate method to the determination of glucurone and also to "galacturone". The free acids were converted to the lactones at 120°C in an autoclave (15 lb. per sq.in.) at pH 1.5 and then alkaline hydroxamate added, the final pH being 1.2 as in the Kaye and Kent method.

On the evidence from the literature, then, this reaction appears to be the most promising for the general estimation of uronic acids, although no critical survey of possible interfering substances has been carried out.

There are a large number of colorimetric methods of uronic acid analysis based on the formation, with a reagent, of coloured compounds from the decomposition products of uronic acids in strong acid solution. These reactions are non-stoichiometric and the analyses therefore depend on the standardisation of the method with a pure uronic acid, either glucuronic or galacturonic acid.

Tollens (188) first used Naphthoresorcinol as a reagent for the determination of galacturonic acid, but it has been shown (189) that the reaction is not specific even when recommended modifications are carried out. It seems that almost every naturally occurring hydroxy compound interferes (190), although a number of recent investigators (191) have used the method when uronic acids only have been thought to be present. It has been suggested that reported colour formation with arabinose and mucic acid is due to impurities (190).

Other similar reactions based on the formation of coloured compounds with many reagents in acid or alkali are known (192). All are subject to a greater or less degree of interference from many other carbohydrate materials.

Anthrone is a reagent of this type, which, with the addition of conc. H_2SO_4, can be used for the estimation of both pentose and hexuronic acids together, although some amino-acids also interfere (193).

Carbazole (dibenzopyrrole) was first used as a qualitative reagent (194) but Dische (195) placed the reaction on a quantitative basis.

Using approx. 85% H_2SO_4 and a 0.1% solution of carbazole he compared the optical densities of uronic acid solutions after heating at 60°C and at 100°C for 90 sec., and found it possible to distinguish between glucuronic and galacturonic acids due to the absorption at 527 mμ being 30 times more intense for galacturonic acid than for glucuronic acid. Sugars do not interfere to any great extent, galactose giving the largest error (196).

Stark (197) used the first Dische method, i.e. heating the mixture for 20 min. at 100°C to determine the total uronic acid content in cotton pectins and compared his results with the 12% HCl decarboxylation method of Whistler, Martin and Harris (80), the results being given as anhydro-uronic acid and corrected, by subtraction, for CO_2 given off by the same weight of cellulose. The results show that, on the whole, the values obtained by the carbazole method are slightly lower than those by decarboxylation.

McCready and McComb (198) made a study of the carbazole reaction and the application of it to the determination of uronic acids in pectins. The authors modified the method and also found that the esterified galacturonic acid gave low results. Most sugars were found to interfere only slightly in the reaction when mixed with a known weight of galacturonic acid. Again it was found that the 19% HCl decarboxylation method gave higher results with pectins of low uronic acid content but with high uronic content the results agreed closely. Glucuronic acid was not estimated in this work.

The estimation of glucuronic acid by the carbazole method in the monomer and polymer form was carried out by Bowness (199) who modified the procedure, taking readings after 2.5 hrs. and 24 hrs.,

and so being able to estimate both glucuronic acid and glucose.

In the estimation of uronic acids in soils, the results obtained by the carbazole method were compared with those of the decarboxylation reaction, by Dubach and Lynch (200). Variable results were obtained by decarboxylation as compared with the carbazole method, but no attempt was made to find the source of the relatively large amount of CO_2 from soil layers.

Meyer and co-workers (25) obtained evidence for the presence of L-iduronic acid in Chondroitin sulphate B by the ratio of optical density by the carbazole method to that with the orcinol method; this was confirmed by hydrolysis and chromatographic studies. The results they obtained show clearly the variation on colour given by different uronic acids. The table shows that, although different uronic acids give similar colour formation in the orcinol test, there is a wide variation in the carbazole reaction. In the orcinol test the samples were heated in 20% HCl with orcinol for 20 min. (202).

TABLE XIX

Colour formation from various uronic acids with carbazole and orcinol in acid.

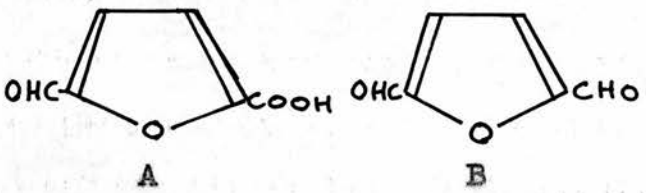
(Colour intensity of D-glucurone = 100)

Compound	Carbazole test	Orcinol test	Carbazole/orcinol ratio
D-glucurone	100	100	1.00
L-gulurone	32	106	0.30
L-iduronic acid	29	130	0.22
D-mannurone	17	128	0.13
D-galacturonic acid	120	127	0.95

These then are the main colorimetric methods of uronic acid estimation. Since both the decarboxylation method and the Kaye and Kent method have been used in this department for a number of years it was decided that a comparative study of the Anthrone method, Carbazole method and the Kaye and Kent method might give interesting results when all three were compared with the decarboxylation method investigated in Part II.

Stutz and Deuel (65) isolated 0.3-0.5% 5-formyl furoic acid from the sulphuric acid decomposition products of galacturonic acid and found this substance gave a colour with carbazole but not with other colorimetric reagents such as naphthoresorcinol.

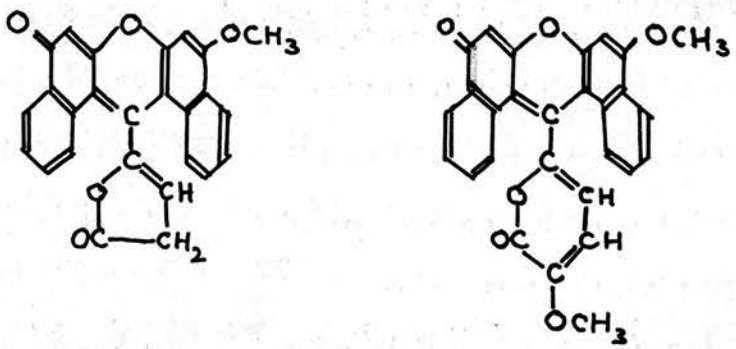
Bowness (203) also showed that furfural and 5-hydroxy methyl furfural gave no substantial colour with carbazole. However, both 5-formyl furoic acid (A) and 2,5-diformyl furan (B) give colours with carbazole but have different spectra; that of the 5-formyl furoic acid is almost identical with the spectra from glucurone and galacturonic acid, hence this is the chromogen in this determination. From the molar extinction coefficients Bowness estimated that 25.8% 5-formyl furoic acid is formed at 60°C under the reaction conditions.



A B

Pentoses and hexoses give a different colour from hexuronic acids when treated with naphthoresorcinol in hydrochloric acid. Guerrero and Williams (204) found that the dye was also formed by the fusion of naphthoresorcinol with glucuronic acid and suggested that

the colour was due to a xanthine or dinaphthyl methane derivative. The structure of two chromogens was suggested by Japanese workers (205) on the basis of spectroscopic studies to be



5-Formyl furoic acid has also been shown to be the chromogen in the reaction of anthrone with uronic acids in conc. H_2SO_4 (206).

EXPERIMENTAL

(1) Kaye and Kent method (185).

2 ml. of a solution containing a known weight of glucurone were pipetted into a flask, 1 ml. of water was added and 2 ml. of a freshly prepared solution of hydroxylamine (made by previously mixing equal volumes of 2M.hydroxylamine hydrochloride and 3.5M.NaOH). The solution was allowed to stand for four minutes and 1 ml. 3.34M. HCl was added followed by 1 ml. $FeCl_3$ solution (0.37% in 0.1N.HCl). All volumes were measured by pipette. The solution was then shaken under reduced pressure to remove the gas evolved. A blank solution was made up at the same time with 2 ml. water in place of the glucurone solution. The optical density of the resulting red solution was read against the blank using a Unicam SP600 spectrophotometer at 5050Å. with calibrated 10 m.m. stoppered silica cells.

A curve of optical density versus concentration was obtained for glucurone and glucose. According to Kaye and Kent the absorption for glucurone alone is too high by the amount of absorption from the reducing end of the molecule. They therefore subtracted the absorption curve for glucose from the glucurone curve and called this the "corrected" glucurone curve. They then used this curve for the estimation of the uronic acid content of polysaccharides.

Since the uronic acid in a polysaccharide will be in the pyranose form, it is necessary to convert this to the ester.

Known amounts of the polysaccharides (approx. 30 mg.) were weighed into glass tubes and 2 ml. 1% methanolic hydrogen chloride

added. The tubes were sealed and heated in a boiling water-bath for 30 min. The contents of the tubes were then each washed into graduated flasks and made up to 50 ml. with water. 2 ml. of each solution were used for each determination which was carried out as for glucurone, the wt. of uronic acid present being read off from the standard "corrected" glucurone curve.

(2) Dische carbazole method (195).

The method described by Dische for the determination of the total uronic acid content of a polysaccharide material was used.

1 ml. of a solution containing 5-100 µg. of uronic acid was mixed, with cooling, with 6 c.c. of concentrated sulphuric acid (Analar grade) and heated for 20 min. in a boiling water-bath. The mixture was then cooled to room temperature and 0.2 ml. of a 0.1% solution of pure carbazole in pure ethanol added, with shaking. After a few minutes a pink colour appeared; the optical density at 530 mµ being measured after 2 hrs. standing at room temperature (22°C), using 10 m.m. silica cells. Conc. H_2SO_4/carbazole was used as blank. Standard curves for galacturonic acid monohydrate and glucurone were obtained.

(3) Anthrone method (196).

2 ml. of a solution containing 100-500 µg. per ml. of uronic acid were placed in a test-tube having a ground glass stopper, and 0.5 ml. of an anthrone solution (2% anthrone in ethyl acetate) were added. 5 ml. concentrated sulphuric acid were added, the solution being gently agitated. Uronic acids give a red colour with a

maximum absorption at 5400Å and 6250Å. This second absorption peak is also given by other sugars so the absorption was measured at 5400Å. The solution was allowed to stand for 15 min. at room temperature before the absorption was determined. In this case the absorption increases steadily with time, no marked maximum absorption being found. Standard curves for glucurone and galacturonic acid were obtained by plotting the optical density against concentration. Amino-acids affect the anthrone reaction in different ways (193), depending on the temperature, heating time and concentration, and some enhance the colour while others have either no effect or depress the colour.

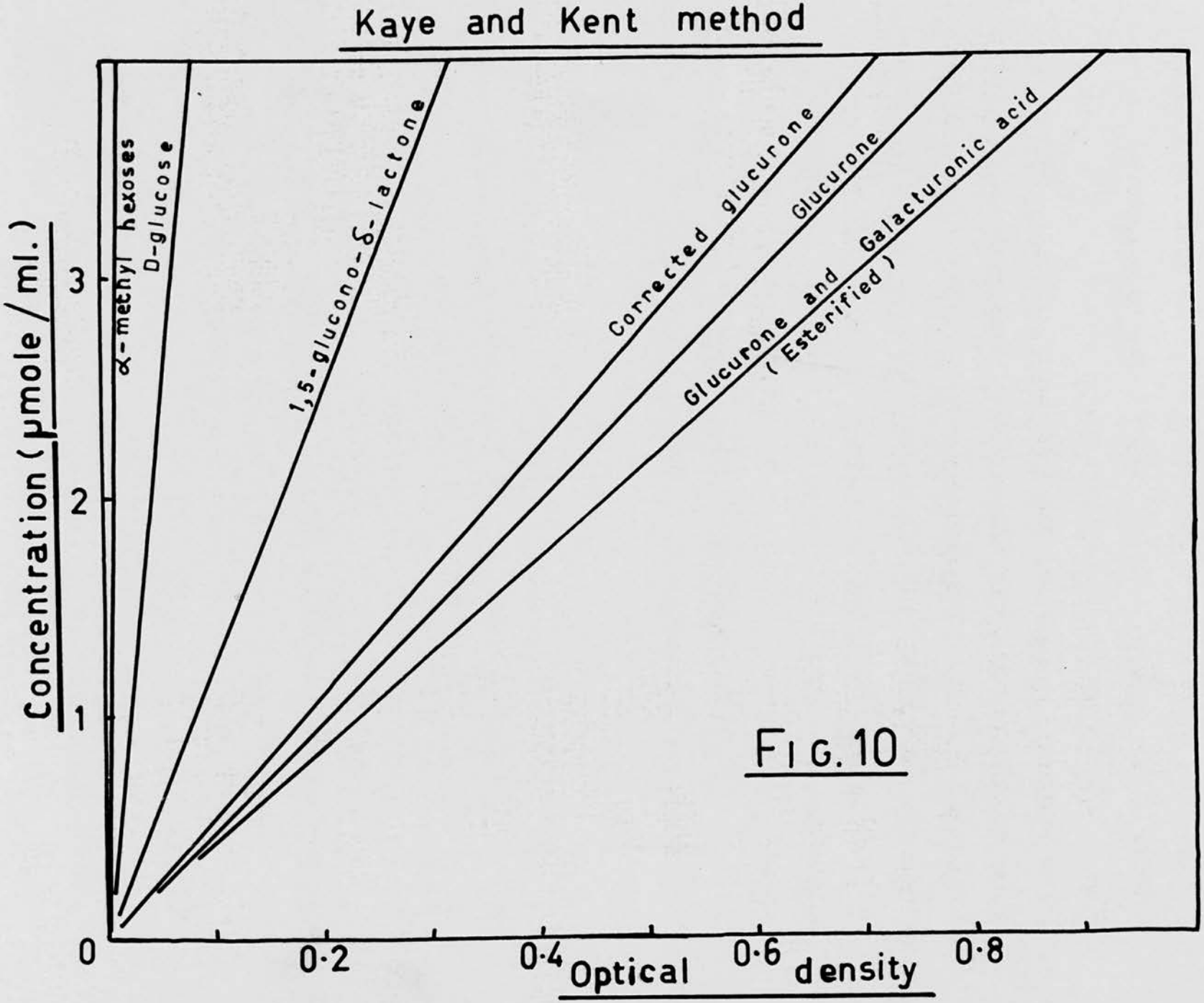

Kaye and Kent method
Concentration (µmole / ml.)
0
1
2
3
0·2
0·4
0·6
0·8
Optical density
α-methyl hexoses
D-glucose
1,5-glucono-δ-lactone
Corrected glucurone
Glucurone
Glucurone and Galacturonic acid
(Esterified)

FIG. 10

EXPERIMENTAL RESULTS

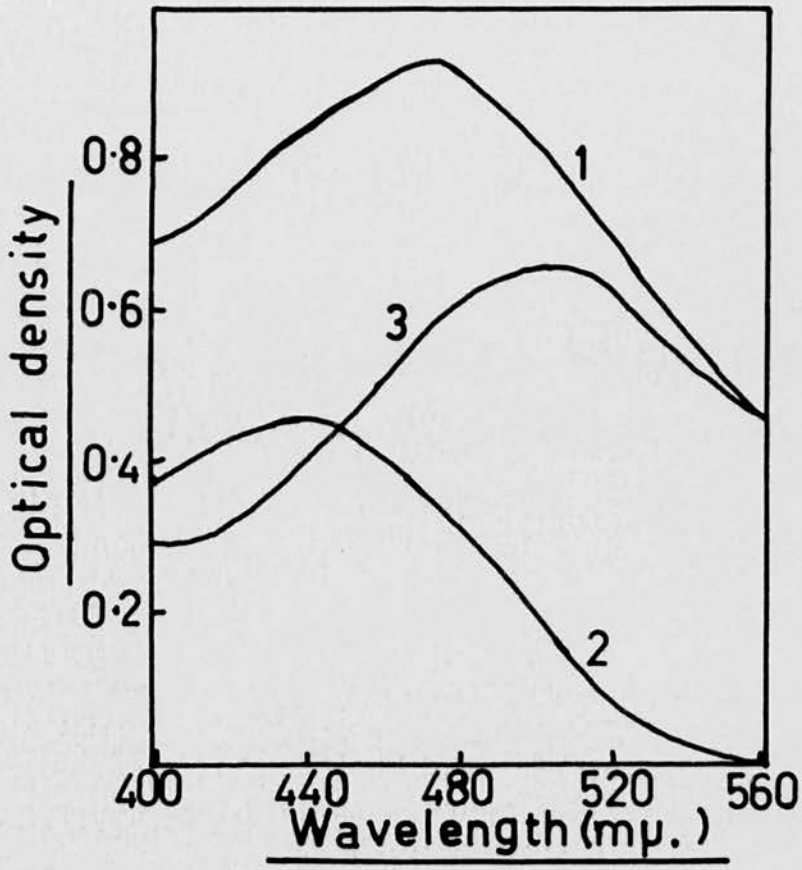
1) Kaye and Kent method.

A standard curve of optical density versus concentration was obtained for pure glucurone, and the "corrected" glucurone curve obtained by subtraction of the curve for glucose to allow for the absorption due to the reducing end-group.

Standard curves for glucurone and galacturonic acid monohydrate were also obtained by esterification carried out as for polysaccharides. If the standard glucurone curve was correct, the esterified and non-esterified curves should be the same; Fig.10 shows the curves obtained. It is seen that the curves for esterified glucurone and galacturonic acid monohydrate are coincident. The standard glucurone curve differs from these quite considerably; it is suggested that this is because of the equilibrium between glucuronic acid and glucurone; the acid form is favoured in alkali and hence in the initial stages of the determination some glucurone will be hydrolysed by this reaction. Lien (187) found that hydrolysis of gluconolactone took place at pH greater than 10; it is also possible that this reaction occurs with glucurone.

Fig. 11(a) shows the plot of optical density versus wavelength. This shows that in the presence of a reducing sugar the uronic acid will give a maximum absorption at 5050Å. Fig. 11(b) gives the plot of optical density versus time. This shows that the colour is stable for only 15 min.

Also shown on Fig.10 are the curves for various sugars. It is seen that the esterified sugars give very little absorption so that



Kaye and Kent method

(a)

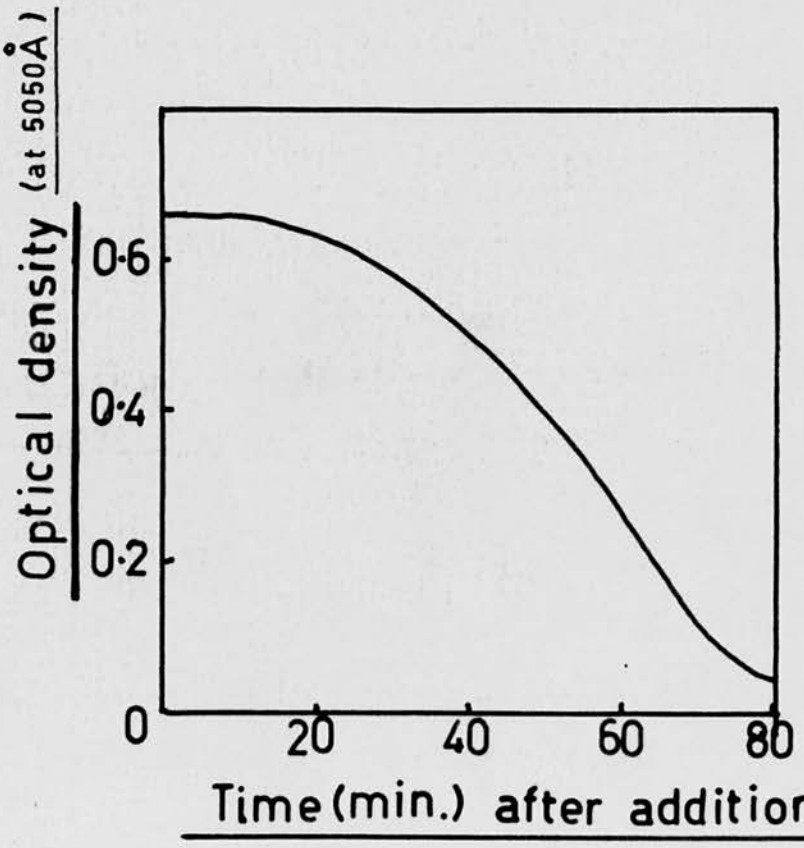
CURVE :

1 : D-glucurone

2 : D-glucose

3 : D-glucurone corrected for D-glucose absorption

FIG. 11



(b)

D-glucurone

they will not interfere in the determination of uronic acid content of esterified polysaccharides. It is suggested from these curves that the esterified glucurone and galacturonic acid curves should be used in the estimation of uronic acid content of esterified polysaccharides, instead of the "corrected" glucurone curve recommended by Kaye and Kent.

This method suffers from high protein interference; consequently, the polysaccharide should be protein-free before a determination is carried out. Since most polysaccharides contain some protein contaminant, the method is generally somewhat inaccurate. Table XX(a) shows the effect of protein on the estimation of galacturonic acid. Weighed amounts of edestine were mixed with a known weight of galacturonic acid monohydrate and esterified as for polysaccharides.

TABLE XX(a)

The effect of protein on the estimation of uronic acid by the Kaye and Kent method.

% protein in mixture	% error in uronic acid content
17.7	+ 6.4
50.3	+ 80.8
90.6	+ 181.0

In this case a positive error is found, the protein increasing the optical density above that expected.

The absorption of sugars is relatively small; but in polysaccharides of low uronic acid content the percentage of sugars present is high and the interference will be proportionately greater.

A range of mixtures of glucose with galacturonic acid monohydrate were made up and estimated after esterification; the results are summarised in Table XX(b).

TABLE XX(b)

The effect of non-uronic acid carbohydrates on the estimation of uronic acid by the Kaye and Kent method.

% composition of mixture		% error in uronic acid content
Glucose	Galacturonic acid	
13.3	86.7	+ 0.1
52.0	48.0	+ 2.5
96.0	4.0	+ 30.1

The use of galactose in place of glucose was found to give a slightly greater positive error; pentoses gave a slightly smaller positive error than glucose.

In the determination of uronic acid content of polysaccharides, accuracy depends on the complete esterification of the carboxyl group. In some cases, however, the polysaccharide was found to be incompletely soluble in methanol/HCl and it was thought that this could lead to errors in estimation. Also, some of the polysaccharides failed to dissolve completely in water, so that, on taking an aliquot of the diluted solution, a large error would occur. Prolonged heating in methanolic hydrogen chloride and the use of stronger acid did not alter the results. The values obtained for various polysaccharides were compared with values obtained by the decarboxylation method already described and are shown in Table XX(c).

TABLE XX(c)

The determination of the uronic acid content of various polysaccharides by the Kaye and Kent method and comparison of the results with the Lefèvre-Tollens decarboxylation method.

Symbols: A = polysaccharide does not dissolve completely in 1% HCl in dry methanol.

B = esterified polysaccharide does not dissolve in water.

Polysaccharide	% Uronic acid		Remarks
	Kaye and Kent method	Decarboxy-lation method	
Bone gelatine (No.188)	14.0	3.5	Contains protein
Gum ghatti	8.5	14.6	A
Alginic acid	65.7	97.1	A, ppt. with Fe^{+++}
Degraded pectin	45.8	58.9	A,B
Hydrolysis residue of Pectin	50.7	68.8	A,B
Polysacc. from Green seaweed	24.4	17.0	A,B, contains protein
Ammonium pectate	48.0	55.0	A, ppt. with Fe^{+++}
Hyaluronic acid (Jensen prep.)	42.2	31.6	A, contains protein
Gum nodule (8) (C. leonense)	15.8	20.0	-

It is seen that, in general, protein increases the apparent uronic acid content, while incomplete solubility and precipitation with the addition of ferric ions markedly decreases the apparent uronic acid content. The Kaye and Kent method is, therefore, not suitable for the determination of uronic acid content of pectins.

This method then is seen to be open to the following criticism. 1) Although this method was put forward as a micro-method (concentrations of 1 μ mole/ml. or 200 μg./ml. can be measured), up

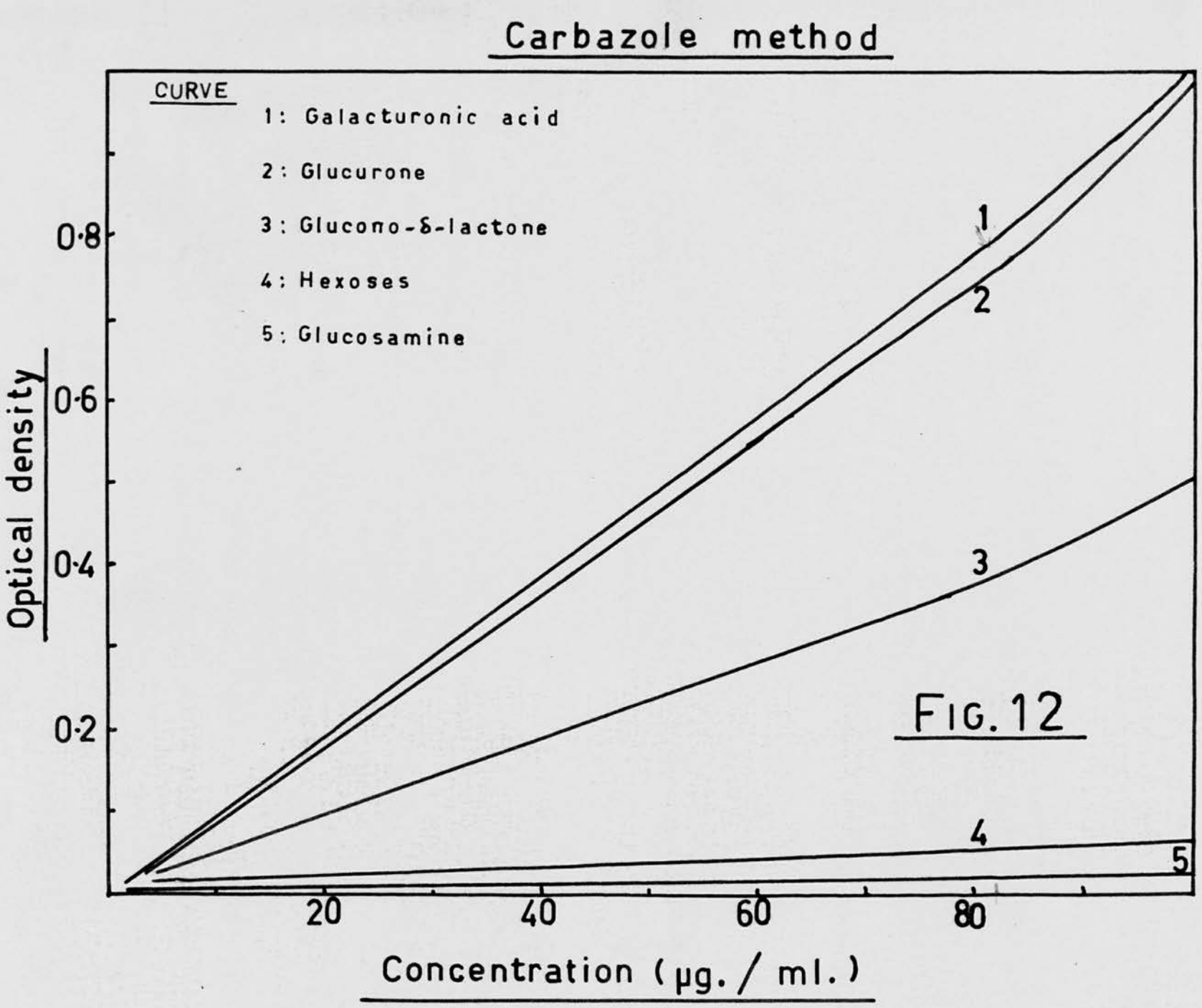

Carbazole method
CURVE
1: Galacturonic acid
2: Glucurone
3: Glucono-δ-lactone
4: Hexoses
5: Glucosamine
Optical density
0·8
0·6
0·4
0·2
1
2
3
4
5
20
40
60
80
Concentration (µg. / ml.)

FIG. 12

to 30 mg. of material are required. This is esterified in 1% HCl in methanol but can subsequently be recovered as the esterified and partially methylated polysaccharide.

2) Four different standard solutions are required and these solutions must be freshly prepared; the use of these solutions also makes the method somewhat tedious.

3) The method is influenced by the presence of protein and by excess of non-uronic acid sugars such as occur in many polysaccharides.

4) Errors have also been found to occur if the material being estimated is incompletely soluble in the 1% HCl in methanol or in the solution when diluted with water. Pectins and alginic acid are also precipitated out when the ferric chloride solution is added, again leading to very large errors.

2) Carbazole method.

Standard curves were obtained for glucurone and galacturonic acid using the method previously described. The curves show a definite divergence, with galacturonic acid giving the greater optical density for a given concentration; this agrees with the findings of Meyer and co-workers (Table XIX, p.195). The curves obtained for the uronic acids along with those for various sugars are shown in Fig. 12. It is seen that glucono-δ-lactone interferes to quite a marked extent and will therefore be estimated as uronic acid in oxidised polysaccharides. However, at low concentrations, sugars do not interfere to any great extent; hexoses give the highest absorption,

with pentoses less. However, errors will occur if large amounts are present. This is shown in Table XXI. Known weights of glucose were mixed with known amounts of galacturonic acid and the absorption measured, the % error being calculated.

TABLE XXI

The effect of non-uronic acid carbohydrate material on the estimation of uronic acid by the Carbazole method.

% composition of mixture		% error in uronic acid content
Glucose	Galacturonic acid	
15.1	84.9	+ 1.1
48.0	52.0	+ 6.3
90.0	10.0	+ 20.1

Under the conditions of the reaction, pentoses gave practically no visible red colour up to a concentration of 80 µg/ml.

Dische found that large errors occurred in the presence of protein, the colour being suppressed. He found a 20% error with a 0.1% protein solution and a 40% error with 0.25% protein solution; these are much higher values than would be encountered in polysaccharide chemistry. However, mixtures of known composition of various proteins with galacturonic acid were made up and the uronic acid content measured by this method. Papain, Trypsin and Lysozyme were used; all showed slightly decreased absorption, but in the quantities used appear to have very little effect. So it was found that in a carbohydrate containing less than 10% protein, the carbazole method could be used. Amino sugars such as glucosamine interfere to

a slightly smaller extent than hexoses.

The limitations set by Dische do still apply. He states that the carbazole reaction "cannot be used for accurate determinations of absolute values for hexuronic acids in polysaccharides because its intensity with some polysaccharides is lower, and with others a little higher, than corresponds to the true content of uronic acid". Also it can be seen from Table XIX that large errors would arise from the estimation of mannuronic acid in alginic acid using a glucurone curve. Also the use of concentrated sulphuric acid in the determination requires great care in the measurement of the optical density of these solutions.

If a large number of determinations are required, 10 min. must be allowed for each. Although the colour is fairly stable, this means that the first determination will be done 2 hr. before the last in a batch of a dozen samples. It is considered that this will lead to errors in the presence of other sugars and proteins whose colour formation increases with time.

Helbert and Brown (193) studied the effect of amino-acids on the anthrone reaction and found that the effect varied for different amino-acids and also on the temperature, heating and standing time and on concentration. These also appear to affect the results for protein interference with the carbazole reaction, as stated above.

However, this reaction is helpful as a guide to the presence of uronic acids in natural products.

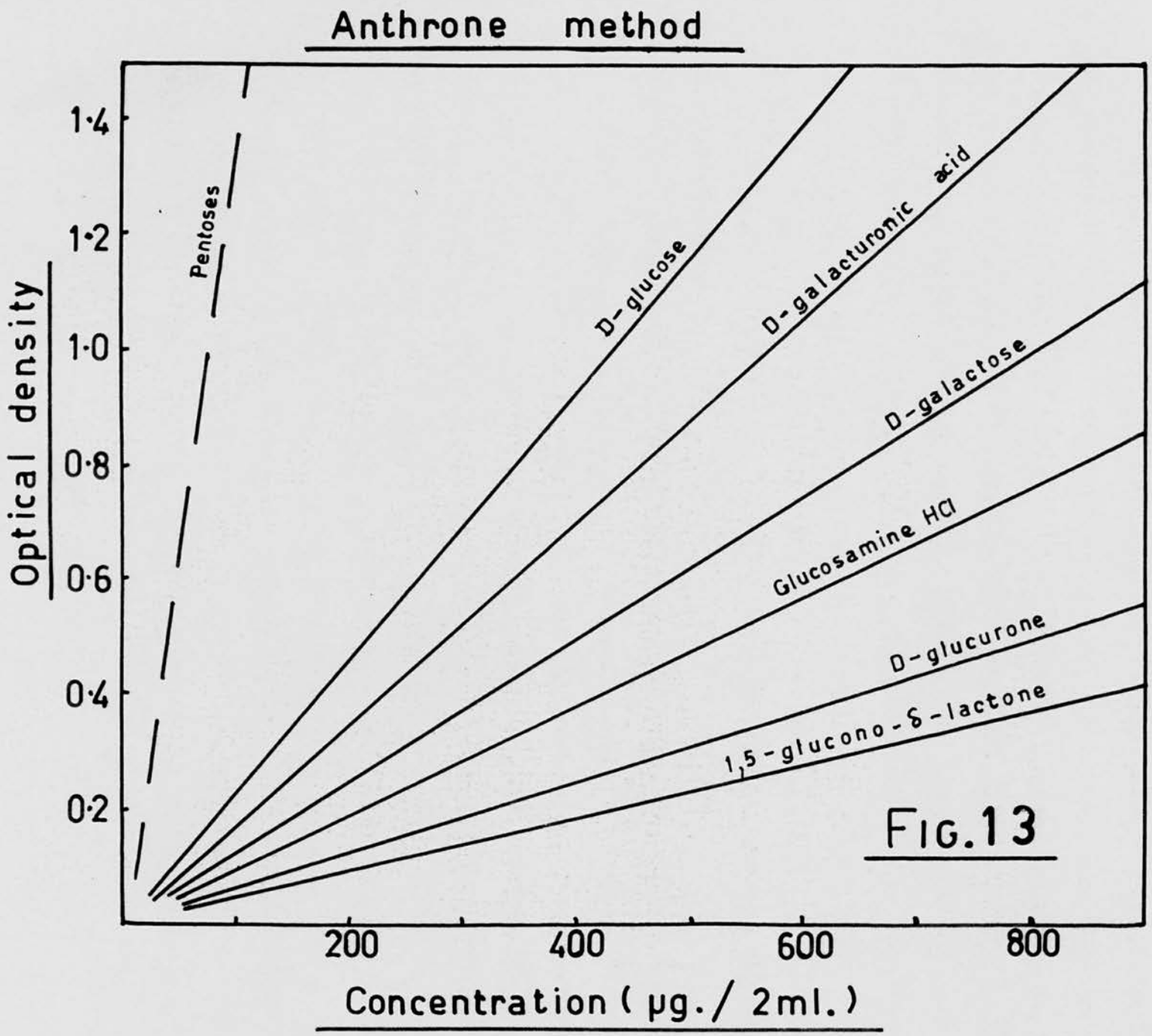

Anthrone method
Optical density
1·4
1·2
1·0
0·8
0·6
0·4
0·2
Pentoses
D-glucose
D-galacturonic acid
D-galactose
Glucosamine HCl
D-glucurone
1,5-glucono-δ-lactone
200
400
600
800
Concentration (µg./ 2ml.)

FIG.13

3) Anthrone Reaction.

This method is generally applicable to the determination of all carbohydrates; but, as can be seen from Fig. 13, each carbohydrate gives a different optical density at a given concentration. However, for fairly pure samples of uronic acids, such as commercial samples, it should be possible to estimate a given uronic acid using the standard curves for glucurone or galacturonic acid.

4) Comparison of the above colorimetric methods with the Lefèvre-Tollens decarboxylation method.

As we have seen, none of the colorimetric methods available for the determination of uronic acid content in a polysaccharide is specific; at best, the colorimetric methods give estimations, not determinations.

From parts I and III of this thesis it is seen that the Lefèvre-Tollens method is also subject to errors from other carbohydrate materials present, but the presence of protein does not appear to affect the estimation, nor does the insolubility of the polysaccharide in water or acid affect the result. Interference from esterified carboxyl and hydroxyl groups, as in some pectic acids, does give rise to methyl chloride but this can be allowed for by the determination of the methoxyl content by a modified Zeisel method (207) and the equivalent subtracted from the total estimation. The carbon dioxide arising from non-uronic acid material can also be allowed for by subtraction. It was decided, therefore, to compare the values obtained by the three colorimetric methods with the decarboxylation method (see Part I) for various uronic acid and

non-uronic acid materials to see which gave the most consistent and probable results. The results are shown in Table XXII. The decarboxylation was carried out as described in Part I, i.e. 2½ hr. in 19% HCl with CO_2-free nitrogen as flow-gas, the CO_2 being absorbed in standard baryta. The results are expressed as % moles CO_2 per mole monomer to make them comparable to the colorimetric values. The commercial samples of uronic acids are as named, the root samples were obtained from the Macaulay Institute for Soil Research, Aberdeen; the Bone Gelatine from the British Bone and Glue Research Institute. Other samples were obtained from members of this Department.

It is seen from Table XXII that there is fair agreement between the colorimetric methods and the decarboxylation reaction for the galacturonic acid monohydrate and trigalacturonic acid samples. However, for the glucurone samples the values of over 100% suggest some impurity which interferes with the colorimetric methods, but is not estimated by the decarboxylation reaction, hence the values are less than 100%. Glucono-δ-lactone interferes to a large extent with the colorimetric measurements, but not to such an extent with the decarboxylation. Melezitose, a trisaccharide, gives high values with the colorimetric method, but small interference with the decarboxylation. As expected, the anthrone method gives high values for the hyaluronic acid, bone gelatine, and the plant root samples, but the carbazole and decarboxylation values agree well for the roots and hyaluronic acid but differ very considerably for the gelatine. For the pectins with high uronic acid content the Kaye and Kent method gives large errors but the carbazole and decarboxylation results are in close agreement.

TABLE XXII

The estimation of uronic acid content of various compounds containing carbohydrates.

Compound	Kaye and Kent	Dische carbazole	Anthrone method	Decarboxy-lation
Galacturonic acid monohydrate				
'Roche'	98.2	98.0	99.0	96.0
'Eastman'	70.5	70.2	71.4	75.7
B.D.H.	86.4	85.8	87.1	88.4
'Light'	76.6	76.2	77.8	80.5
Trigalacturonic acid	87.9	87.5	88.0	92.4
Glucurone				
'Roche'	119.8	118.7	119.9	78.9
'Corn Products' 824	101.1	100.1	101.5	91.1
'Gen. Biochemicals'	117.2	116.6	117.3	90.9
Glucono-δ-lactone	51.7	51.4	62.1	9.9
Melezitose	14.8	15.0	17.0	1.6
Hyaluronic acid	42.2	34.8	57.6	31.6
Bone gelatine (No.112)	12.2	12.3	27.6	2.3
Plant root samples				
1.	7.6	8.2	25.4	6.6
2.	8.3	9.7	27.6	7.1
3.	6.4	7.6	24.3	6.3
4.	6.8	8.0	24.2	7.2
Ammonium Pectate	51.4	77.4	77.8	74.1
Pectin	42.5	60.2	67.8	57.9

It seems fairly clear, that for estimation of uronic acid content in polysaccharide materials of unknown composition the decarboxylation method (in conjunction with a methoxyl determination) will give the most accurate results. The disadvantages, as compared with the colorimetric method, are that

1) A large sample of up to 30 mg. polysaccharide is required.

2) Two and a half hours are required for the completion of the decarboxylation.

3) The apparatus in which the estimations are carried out can only be made by a skilled glass-blower, but once made is very robust.

On the other hand, the colorimetric methods are so susceptible to interference as to be totally unreliable for uncharacterised materials. Also the decarboxylation reaction can be left alone for 2½ hr., and the time required for titration and cleaning out of apparatus is less than half an hour, so that one determination can be carried out every three hours. There is, then, very little difference in actual working time for the two types of method for less than six uronic acid estimations, especially if two decarboxylation apparatus are available.

As far as the colorimetric methods themselves are concerned, the carbazole reaction is the most useful for routine determinations, provided the carbazole solution, (which deteriorates very quickly), is freshly prepared. However, the use of concentrated sulphuric acid in optical instruments is not recommended.

PART VII.

Discussion of the mechanism of decarboxylation of Uronic acids in mineral acid.

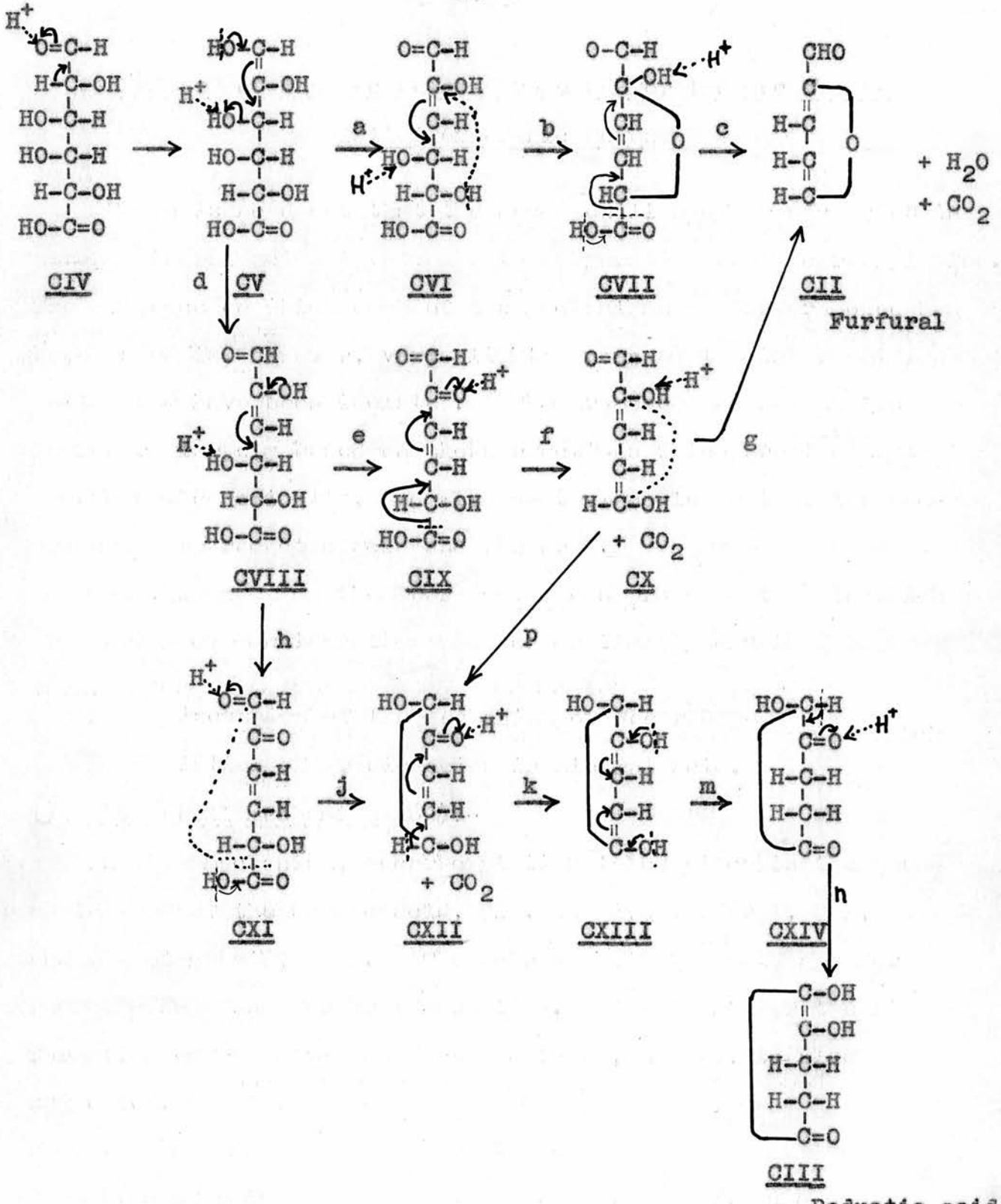
Summary of the mechanisms proposed for the decarboxylation of uronic acids

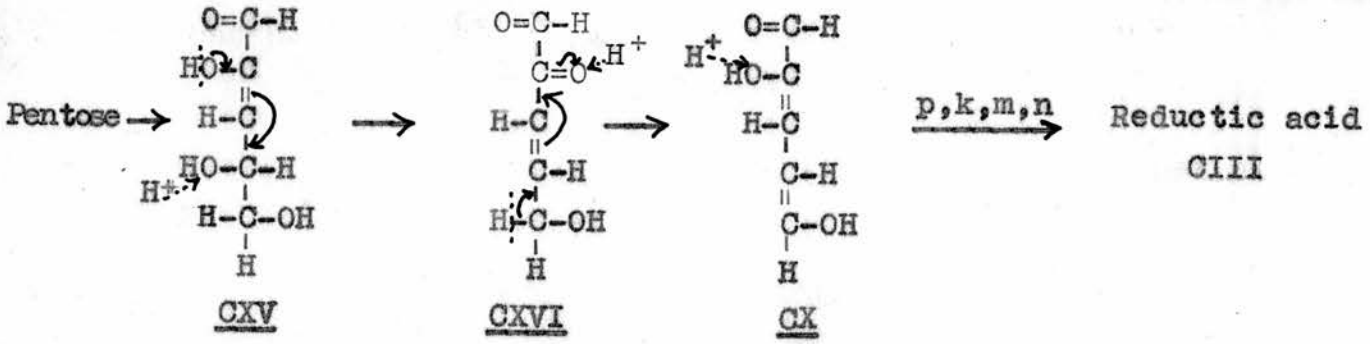
There is no doubt that the decomposition of uronic acids in strong mineral acids involves both dehydration and decarboxylation. The evidence on which each of the mechanisms so far proposed has been based is, however, very slight: none of the intermediates postulated have been isolated. The proposed mechanisms are therefore largely based on those reactions which are the most probable theoretically, and also on the knowledge that the end-products are reductic acid and furfural.

In this section the four mechanisms proposed to date which are worthy of consideration will be critically examined in turn.

1) The Isbell mechanism (69):-

In this mechanism, decarboxylation takes place in the open-chain form of the uronic acid, CIV, to give furfural, CII, and reductic acid, CIII. The scheme shows two pathways for reductic acid and furfural formation. A pathway for the formation of reductic acid from pentoses, LXXXIV, is also suggested.


CIV
CV
CVI
CVII
CII
Furfural
+ H2O
+ CO2
CVIII
CIX
CX
+ CO2
CXI
CXII
CXIII
CXIV
CIII
Reductic acid
a
b
c
d
e
f
g
h
j
k
m
n
p

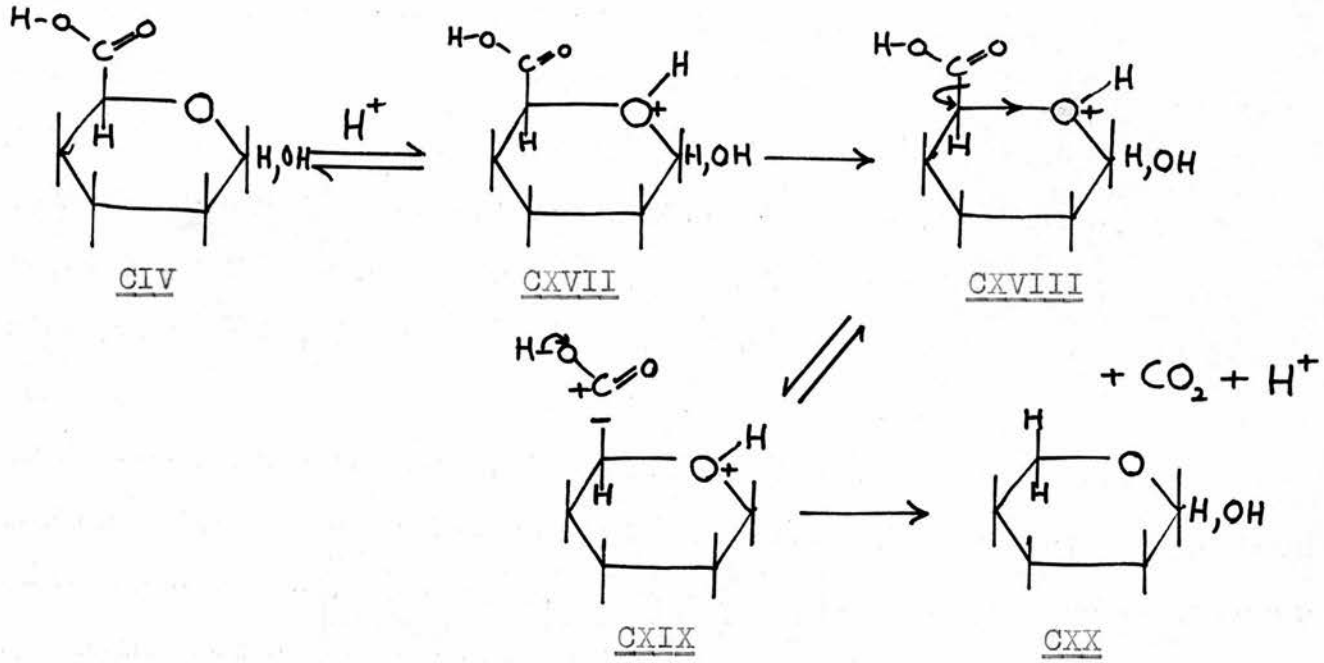

Pentose
CXV
CXVI
CX
p,k,m,n
Reductic acid
CIII

In the uronic acid mechanism step a is facilitated by the equilibrium between the enediol and aldehydic forms; however, this tends to oppose step b. The alternative step e is more probable with water elimination between carbons-4 and -2. Step c, the removal of the hydroxyl group adjacent to a cyclic structure and aldehydic group, is rather unlikely because of the low electron density on the hydroxyl oxygen atom. Step f, the decarboxylation of a β-γ-unsaturated acid, is quite possible, although modern workers give a cyclic intermediate and hence the product should have H-C-H on carbon-3. However, dehydration of CX would probably occur by proton attack on carbon-5 rather than on the low electron density carbon-2 hydroxyl group - furfural would still be the product.

The formation of reductic acid by the proposed mechanism is quite sound; the formation of a carbon-carbon bond during decarboxylation seems the most probable mechanism. A similar mechanism was also suggested by Aso (208).

2) The Huber (70) and Machida (71) mechanism:-

These workers suggested that the decarboxylation took place from the pyranose ring form with the formation of the expected pentose.



The evidence for this mechanism is:-

a) the kinetic data for the reaction indicate a first-order reaction (83)(70)(62).

b) heterocyclic aromatic α-carboxylic acids are readily decarboxylated.

c) hexaldonic acids, with no hemiacetal ring, decarboxylate slowly.

The evidence against this mechanism is:-

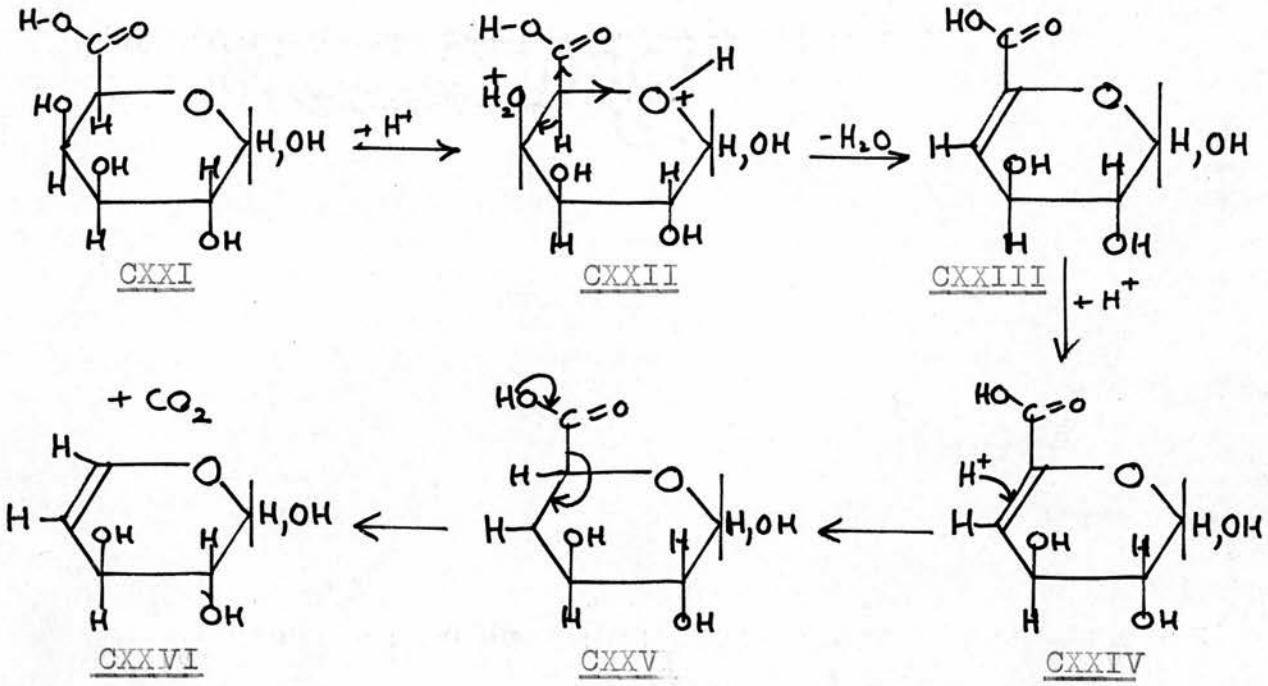
a) the amount of pentose isolated is very small (149)(70).

b) in the acid decarboxylation of uronic acids there is more reductic acid and less furfural formed than in the case of the pentose decomposition (99)(65).

c) 5-keto- and 2-keto-hexonic acids and their lactones decarboxylate easily although they do not exist in the hemiacetal ring form (69).

3) The Zweifel mechanism (140):-

Zweifel suggests the formation, as intermediate, of a cyclic α-β-unsaturated uronic acid giving rise to an unsaturated pentose. The formation of an oxonium complex facilitates the elimination of water from the molecule to give the α-β-unsaturated acid. This is followed by the addition of a proton to the double bond and hence decarboxylation to give an unsaturated pentose. The latter can then ring-open and dehydrate to give both furfural and reductic acid.



The evidence for this mechanism is:-

a) α-β-unsaturated carboxylic acids easily decarboxylate under certain conditions.

b) an α-β-unsaturated aldobiouronic acid, obtained from enzymic attack on hyaluronic acid, was easily decarboxylated; the same compound after hydrogenation did not.

c) 2,3,4-trimethyl galacturonic acid slowly decarboxylates (70).

d) the two mechanisms explain the greater yield of reductic acid from galacturonic acid as compared to that from pentose.

e) the mechanism also explains the easier decomposition of uronic acids, in contrast to pentoses, in dilute mineral acid.

Evidence against this mechanism:-

a) the addition of the proton to the double bond is contrary to the -I effect of the oxonium ion.

b) the ability of the carbonium ion of the uronic acid to exist is very much smaller than the resonance stabilised carbonium ion of cinnamic acid.

c) the existence of the hemiacetal form of the uronic acid in hot strong acid solution, as a stable entity, is somewhat doubtful.

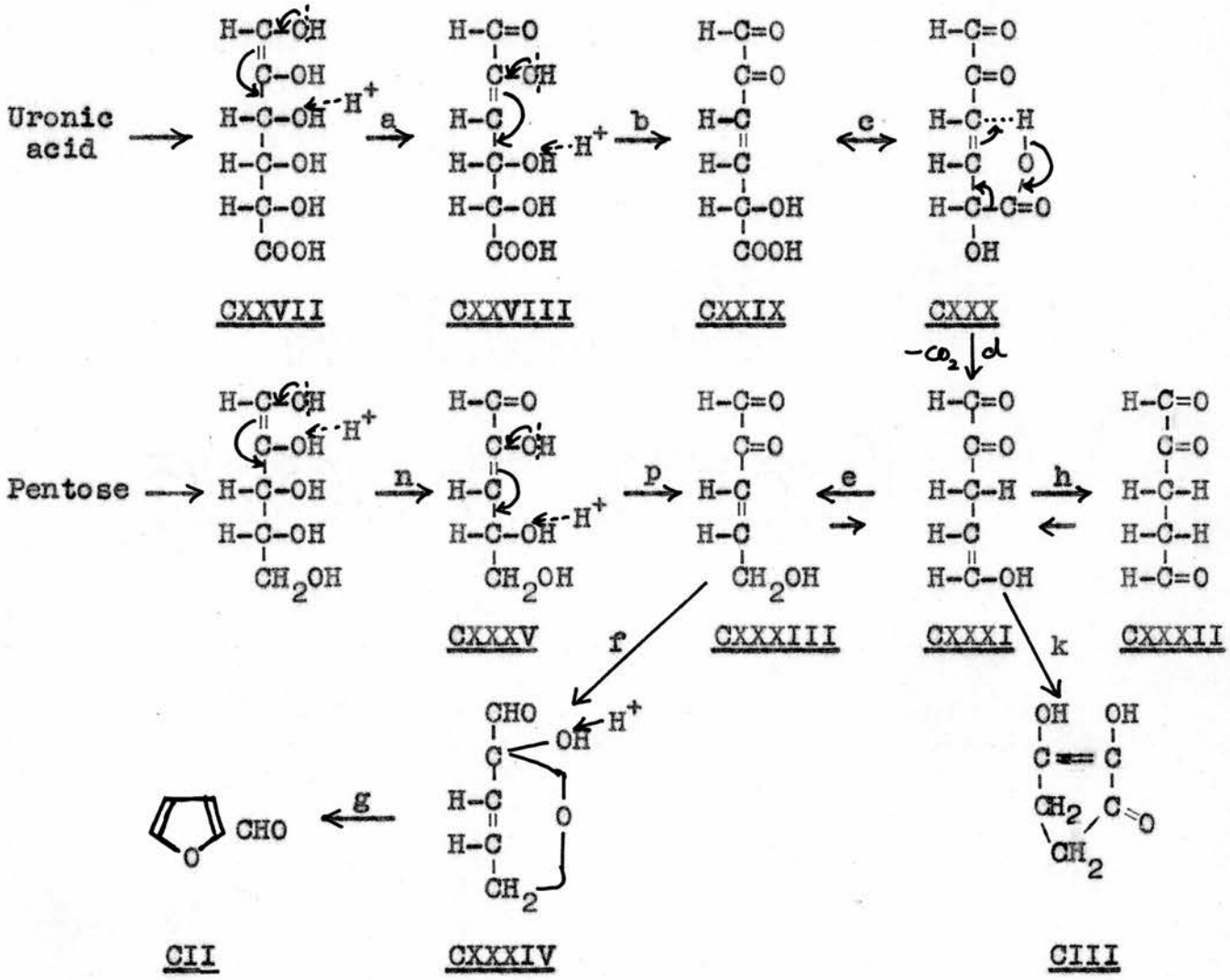
d) no unsaturated pentose has been isolated.

e) 5-keto- and 2-keto-hexonic acids and their lactones decarboxylate easily although they do not exist in the hemiacetal ring form.

f) the unsaturated 'bacterial polysaccharide' quoted by Zweifel did not decarboxylate as easily in weak acid as glucurone (65).

4) The Stutz mechanism (65):-

Decarboxylation occurs from the open-chain form, the initial dehydration step being that of the Isbell mechanism (p.213).



Evidence for this reaction mechanism is:-

a) Compound CXXIX is analogous to the compound formed in the acid dehydration of tetramethylglucoseen which was identified as the osone, LXXX (173)

b) The CO_2 elimination is the well known β-γ-unsaturated carboxylic acid decarboxylation mechanism. According to Stutz this reaction is not catalysed by H_3O^+, the rate only increases with increase in temperature.

c) Compound CXXXI is formed when CO_2 is eliminated and can give CXXXII, CXXXIII or other tautomers. Stutz suggests that CXXXI will be the most likely to give reductic acid since it requires least rearrangement. He goes on to mention that Aso (208) proposed the same compound (or at least a tautomer) in the formation of 3-keto-pyridine compounds from uronic acids in acid solution in the presence of nitrogenous compounds.

d) Compound CXXXIII can form furfural by hemiacetal ring formation, and can also be formed from pentoses. This, having conjugate double bonds, will tend to be more stable than CXXXI and therefore the tautomeric equilibrium will favour CXXXIII and furfural formation.

e) The mechanism therefore explains why uronic acids give a greater yield of reductic acid than do pentoses.

f) It is suggested that one or the other of the tautomeric forms, CXXXI or CXXXII, will be favoured by change in acid strength; hence, depending on the form preferred, more or less furfural or reductic acid will be formed.

Criticism of the above mechanism and evidence can be set out as follows.

a) All the experimental work and discussion given by Stutz indicates

that the stability of the hemiacetal ring governs the rate of reaction as well as the type and position of the substituents; the rate-determining step is the breaking of this hemiacetal ring. The mechanism, however, seems to suggest that neither this nor the conformation of the ring affect the mechanism, although Stutz makes no assumption about the rate-determining step.

b) The intermediate, CXXXI, has not been isolated from uronic acid decomposition products. The formation of an analogous compound from methylated glucoseen only proves that such an intermediate is possible.

c) The rate of decarboxylation of uronic acids does definitely depend on the mineral acid concentration as well as on temperature.

d) Aso (208), in his syntheses of 3-keto-pyridines, used the appropriate furfural derivative as starting material. 3-Hydroxy-pyridine was produced from a number of carbohydrates and nitrogenous compounds but Aso assumed that the reaction first took place with the furfural or derivative formed, not with the intermediates preceding furfural.

e) Since both tautomeric forms are of roughly the same structure it is difficult to see why a difference in acid strength should affect one more than the other, consequently the mechanism does not explain why more furfural is formed the more strongly dehydrating are the conditions; the reverse would appear to be the case from the structures given.

f) The formation of a hydroxy-furfural by cyclisation between a keto group and secondary hydroxyl group, as in step f, is quite unusual, although it was suggested by Wolfrom et al (161); much more probable is a cyclisation between two hydroxyl groups on carbons-2 and-5 as in the Isbell mechanism.

g) The isolation of the osone from the glucoseen decomposition implies that this type of structure has a relatively high stability; therefore, it should decompose at a rate slower than the rate of decarboxylation and so its concentration should increase; further attempts at isolation should therefore be made.

h) Stutz was unable to detect any reductic acid from arabinose in 3.5 N.H_3PO_4 (the best medium for reductic acid formation from hexuronic acids), although Reichstein and Oppenauer (99) isolated 0.05 g. of reductic acid from the decomposition of 40 g. xylose.

j) The final formation of furfural depends on the attack of a proton on a hydroxyl group attached to carbon-2. This attack is possible, giving 1,4-elimination, but will tend to be opposed by the electron-attracting aldehydic group. A much larger variety of products would be formed with this intermediate than just furfural (214); further investigation on the products in solution is required.

k) The formation of reductic acid from CXXXI is not clear; presumably rearrangement is followed by cyclisation between the aldehydic group and carbon-5 as in the formation of reductic acid from pentose by the Isbell mechanism (p. 213). This means

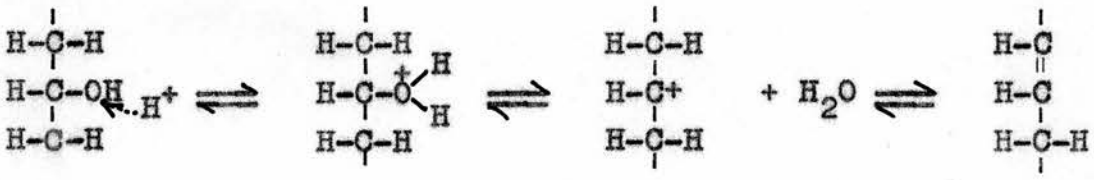
of cyclisation seems much more improbable than the cyclisation after decarboxylation shown in the Isbell mechanism.

It can be seen from the above that none of the mechanisms is entirely satisfactory; all explain the reactions in part. However, since the reaction is so complicated, a final result will only be obtained when a complete quantitative analysis of all the reaction products is made.

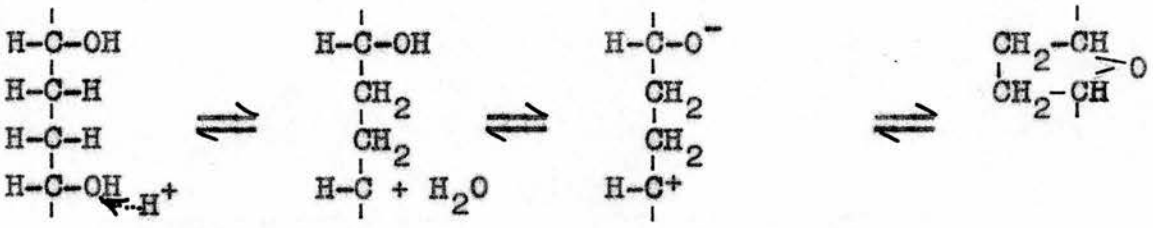
DISCUSSION

The following reactions are postulated in carbohydrate chemistry.

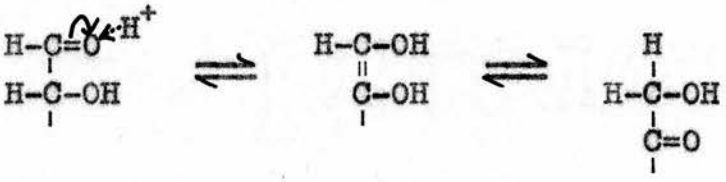
1) Dehydration



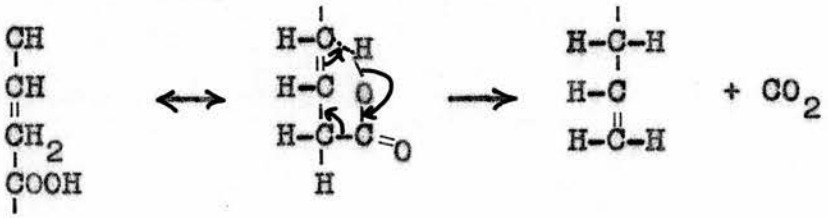
2) Cyclisation



3) Aldehyde-enediol-ketone-tautomerism



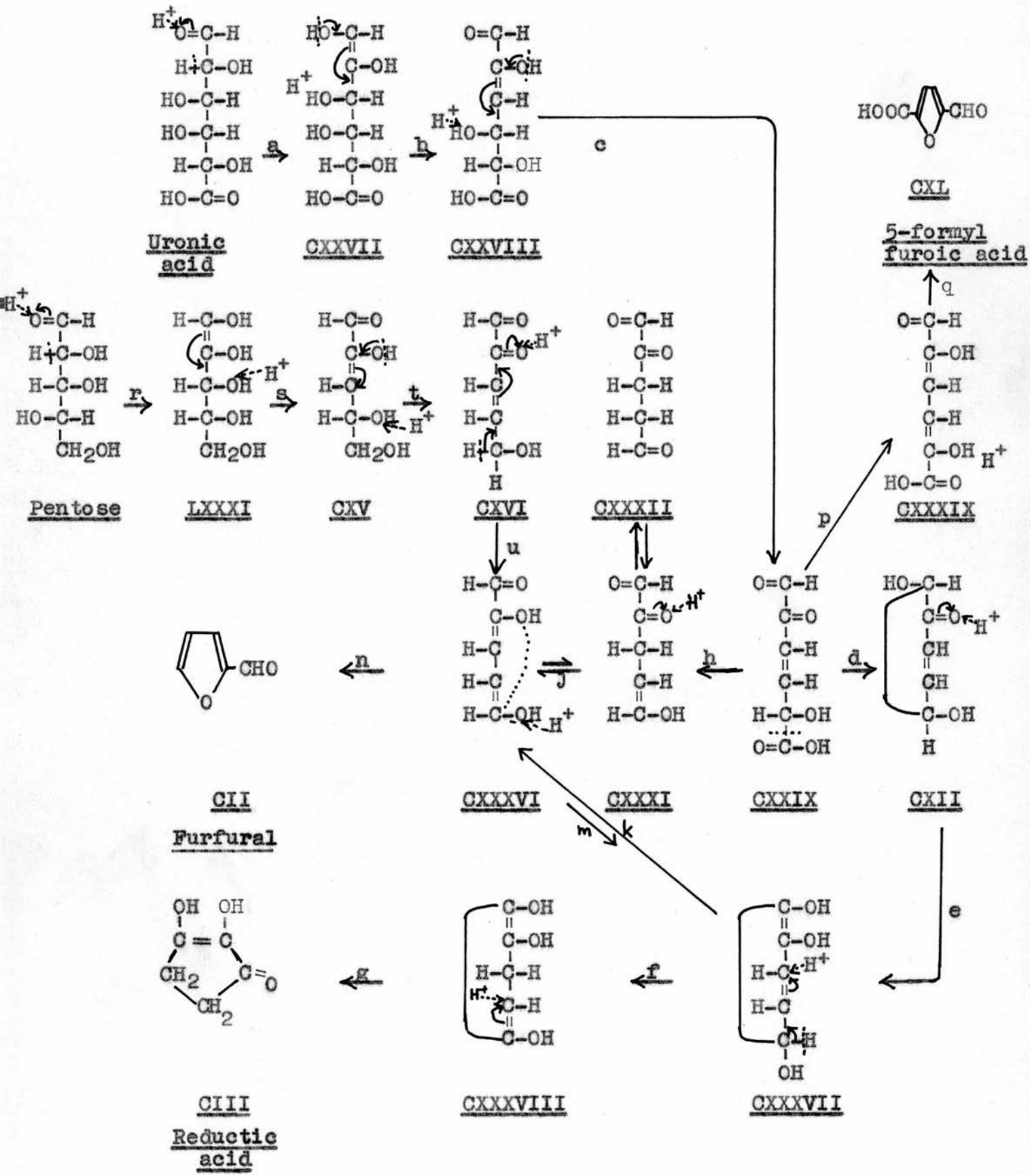
4) β-γ-unsaturated acid decarboxylation



The above will be referred to as mechanisms (1) (2) (3) and(4).

It is suggested that the mechanism proposed below fits all the facts known on the decarboxylation of uronic acids. The position and environment of the hydroxyl on carbon-3 will determine the ease with which a proton attacks the uronic acid and also the ease with which the water molecule is eliminated to give an unsaturated molecule.

The formation of furfural, reductic acid and 5-formyl furoic acid are all explained as well as the formation of furfural and reductic acid from pentoses. The initial stages are the same as those suggested by Isbell (69) and Stutz (65), but the decarboxylation and later steps differ in that they are one stage reactions requiring a single step. (Isbell requires the simultaneous attack by two protons on the molecule: this is unlikely.)


Uronic acid
CXXVII
CXXVIII
CXL
5-formyl furoic acid
Pentose
LXXXI
CXV
CXVI
CXXXII
CXXXIX
CII
Furfural
CXXXVI
CXXXI
CXXIX
CXII
CIII
Reductic acid
CXXXVIII
CXXXVII
a
b
c
d
e
f
g
h
j
k
m
n
p
q
r
s
t
u

Evidence for the above mechanism:-

1) From the evidence given in Part IV that hexoses and pentoses are present as the open-chain enediol form in strong acid, it is probable that uronic acids also exist in that form in strong acid.

No compounds containing a six-membered oxygen heterocyclic ring have been isolated; this is further evidence for decarboxylation in the open-chain form.

Since epimeric hexoses and pentoses decompose at different rates, and since saccharic acids and hexitols only dehydrate (or decarboxylate) slowly, the aldehydic group (in the enediol form) must exert a significant effect on the dehydration reaction. The variations in rate of decarboxylation for various uronic acids must, therefore, be explained in terms of the position of the hydroxyl groups.

2) Stutz (65) compared the rates of decarboxylation of many uronic acid materials in 0.01N.HCl and in 1.75N.HCl. However, since he made no allowance for the methyl chloride formed from the methylated uronic acids, his findings should be accepted with reservation. He found that methylated uronic acids decarboxylate slower than the parent uronic acids. 2-Amino-galacturonic acid decarboxylates faster than galacturonic acid in 0.01N.HCl but slower in 1.75N.HCl. This is due to the formation of a positive charge on the amino-group, so repelling other protons and hence stabilising the molecule. The above would be an acceptable explanation for the open- as well as the closed-chain form; 1-amino-glucuronic acid is stable under acid conditions (201).

3) All the uronic acid materials decarboxylated show approximately the same dependence on the Arrhenius equation, thus indicating the same reaction throughout. This, in conjunction with the dependence on H_o, the Hammett acidity function, (see Part I), rules out the possibility that the rate-determining step is hydrolysis of the lactone ring, e.g. in glucurone, which would involve the presence of a water molecule in the transition state, i.e. all the monomer acids, at least, are in the straight-chain form. The results indicate that this is also true of alginic acid in which case hydrolysis must precede decarboxylation.

4) The log of the rate constant for the decarboxylation reaction is proportional to H_o. This indicates that the rate-determining step involves either (a) the decomposition of a protonated molecule as in an A-1 reaction, or, (b) the addition of a proton to the substrate as in an $A\text{-}S_E2$ reaction. These two requirements are satisfied by all the four mechanisms previously discussed as well as by the above scheme.

5) The values of the entropy of activation (Part I) indicate that an A-1 reaction is the most probable. In this case the rate-determining step is the decomposition of the protonated molecule as in step (b). The rate will then depend on the strength of the C-OH bond on carbon-3, as well as on any substituents in the molecule.

6) Although reductic acid contains an enediol, it seems unlikely that the amounts in which it is formed depend on the dehydration properties of the mineral acid, since reductic acid contains the same

number of oxygen atoms (presumably derived from hydroxyl groups) as does the furfural precursor. The variation of amount of reductic acid formed with the mineral acid used must, therefore, depend on the effect of that acid either on (a) the decarboxylation step or (b) the cyclisation step in the reaction, or both.

7) Since the yield of CO_2 is quantitative, any polymerisation must take place after decarboxylation, hence the predecarboxylation intermediates must contain structures which will not polymerise.

8) The actual intermediate which decarboxylates has not been isolated, but it can be assumed to be a β-γ-unsaturated carboxylic acid, such as CXXIX, formed by dehydration of the uronic acid.

9) Two pathways are proposed for the decarboxylation:-

(a) by the β-γ-unsaturated carboxylic acid decarboxylation mechanism, (mechanism 4), to give a straight-chain intermediate leading to furfural.

(b) decarboxylation and cyclisation as one step to give a carbocyclic intermediate leading to reductic acid.

The following evidence supports the above postulates:-

(i) Under certain conditions, the amount of reductic acid formed from uronic acids increases and the yield of furfural decreases; this suggests competitive formation from the same intermediate.

(ii) Since this decarboxylation step is the only point during the reaction when a carbon-carbon bond is broken, (so effectively giving a lone pair of electrons on carbon-5 which can then either add a

proton, or attack another carbon atom), it seems the most probable point at which a cyclisation could take place.

(iii) The formation of a carbon-carbon bond by cyclisation of an aldehydic end-group to an unsaturated carbon atom (as in step m) to give reductic acid is very unlikely (the reverse step k is much more probable). Nevertheless, a small amount of reductic acid could be produced from pentoses by step m.

(iv) The decrease in yield of reductic acid with increasing mineral acid strength can be explained as follows:- The more concentrated the acid, the greater the tendency for a proton to attach itself to the lone pair of electrons formed on carbon-5 by decarboxylation, hence the preferential formation of furfural. The production of reductic acid should therefore increase with decrease in mineral acid concentration. Increased acid concentration will also facilitate the attack of a proton on carbon-3 in the β-γ-unsaturated acid, so accelerating the decarboxylation and increasing the yield of furfural.

10) In concentrated H_2SO_4 at 90°C and in other strongly dehydrating agents, 5-formyl furoic acid is formed in isolable amounts from uronic acids (65). In boiling 12% HCl, and in dilute acid, practically no 5-formyl furoic acid was detected. Cyclisation between the hydroxyl formed on the carbonyl of carbon-2 (compound CXXIX) would lead directly to this result, provided this attack was faster than the decarboxylation steps h and d. So step p is faster than either h or d; the cyclisation step q will also be very fast.

11) The reductic acid formation from pentoses can be compared to the formation of 2-methyl-3,8-dihydroxy-chromone (208) or 5-formyl furoic acid (65) from uronic acids (i.e. as a side-reaction). This is especially so since Stutz was unable to detect reductic acid in the decomposition of arabinose under conditions which gave good yield from uronic acids.(Table IV p. 43)

12) Since the furfural yield from pentoses approaches theoretical, and that from uronic acids is much smaller, one or more of the intermediates formed from uronic acids must decompose to give polymeric materials. This could occur with compound CXXXI (or tautomers of this such as CXXXII). CO_2 continues to be liberated even after the theoretical yield of CO_2 has been evolved. This could be from the decomposition of the polymeric materials mentioned above.

13) Reductic acid is fairly stable in acid solution. No furan is formed and hence it can be assumed that no furfural is formed by destruction of reductic acid itself.

14) CXXXVI and CXXXVII are both fully conjugated compounds and so will be more stable than the other intermediates. They are considered to be the intermediates common to the pathways of reductic acid formation from pentoses, and of furfural formation (at the expense of reductic acid formation) from uronic acids.

SUMMARY

SUMMARY

(1) Kinetic studies of the decarboxylation of uronic acids.

(a) A linear relationship between the rate of decarboxylation and uronic acid concentration was found.

(b) From the relationship found between temperature and rate of decarboxylation, the Arrhenius activation energy, frequency factor and entropy of activation were determined for some uronic acid monomers and polymers and also for some related materials.

(c) An investigation of the relationship between acid concentration and rate of decarboxylation of various uronic acid materials revealed a linear relationship of rate to the Hammett acidity function; this permitted deductions concerning possible mechanisms.

(d) An investigation of the rate of decarboxylation of uronic acids in water was made.

(e) Decarboxylation of uronic acids labelled with carbon-14 on the carboxyl group gave rate constants in agreement with those found titrimetrically in (b) and (c) above.

(2) Investigation of analytical methods for the determination of uronic acids.

(a) A comparison of the use of oxygen, nitrogen and hydrogen as scrubbing-gas in the analysis of uronic acid was made; nitrogen was found to be most satisfactory.

(b) By consideration of the results found in the preceding sections, deductions were made concerning the best analytical conditions for uronic acid determination, i.e. 19% HCl for two and a half hours with CO_2-free nitrogen as scrubbing-gas.

(c) Attempts to decarboxylate uronic acids by heating at various temperatures gave varying results. Large yields of CO_2 were also obtained from hexose and pentose sugars and the thermal decarboxylation method is not recommended.

(d) The three colorimetric methods most commonly used for the estimation of uronic acids were studied and found to give results which compared unfavourably with those obtained by acid decarboxylation.

(3) The effect of catalysts and antioxidants on the decarboxylation of uronic acids and related materials.

(a) The catalytic effect of metal ions on the rate of decarboxylation of uronic acids in aqueous solution was studied; the rates of decarboxylation were in the following order:-

$Zn^{++} > Co^{++} > Ni^{++} > UO_2^{++} > Mg^{++} > Ca^{++} > Tl^{+} > Ba^{++}$.

Some metals gave overoxidation, others had no effect on rate.

The catalytic effect of various metal chlorides, sulphates and borates was also investigated.

(b) The effect of traces of ferric ions on the rate of decarboxylation of uronic acid and hexose in 19% HCl was investigated; a large increase in yield of CO_2 was found.

(c) In an attempt to suppress the liberation of CO_2 from undesirable side reactions, the effect of a large variety of antioxidants on the rate of liberation of CO_2 from hexoses in 19% HCl was investigated; all decreased the amount of CO_2 formed. The gaseous products from reactions of these antioxidants in 19% HCl were investigated by infra-red spectroscopy; some gave products which interfered with the estimation of CO_2.

The effect of selected antioxidants on the rate of decarboxylation of galacturonic acid in 19% HCl was studied; all decreased the yield of CO_2.

(4) Investigation of sources of CO_2 and other volatile products from materials containing non-uronic acids.

To find which of the substances commonly found in uronic acid materials interfered with the uronic acid determination, the formation of carbon dioxide from non-uronic acid materials was quantitatively investigated. The results were compared with those found by previous workers.

The gaseous products from all the non-uronic acid materials used were trapped in liquid oxygen and analysed qualitatively by infra-red spectroscopy.

(5) Mechanism of decarboxylation of uronic acids.

The four mechanisms put forward by previous workers to explain the facts known about uronic acid decarboxylation were critically examined; a new mechanism, based on previous evidence and evidence obtained in the present investigation, is proposed.

BIBLIOGRAPHY

BIBLIOGRAPHY

Chem. Abs. refers to American Chemical Abstracts.

(1) P.A.J. Gorin, A.S. Perlin, Canad.J.Chem., 34, 693 (1956)

(2) C.L. Mehltretter, Adv. Carbohydrate Chem., 8, 231 (1953)

(3) J.W. Green, in Pigman, The Carbohydrates, Academic Press Inc., New York, 1957, pp. 299-366

(4) R.L. Whistler, C.L. Smart, Polysaccharide Chemistry, Academic Press Inc., New York, 1953

(5) S. Bayne, Ann.Reports, Chem.Soc.,London, 55, 363 (1958)

(6) M. Ameyama, K. Kondo, Bull.Agric.Chem.Soc.Japan, 22, 271 (1958); Am.C.A., 52, 20408 (1958)

(7) M.H. O'Dwyer, Biochem.J., 28, 2116 (1934); Am.C.A., 29, 3827 (1935)

(8) E.L. Hirst, J.C.S., p. 70, (1942)

(9) G.O. Aspinall, I.M. Cairncross, A. Nicolson, Proc.Chem.Soc., p. 270 (1959)

(10) E.G.V. Percival, S.K. Chanda, Nature, 166, 787 (1950)

(11) A.C. Neish, Canad.J.Biochem.Physiol., 36, 187 (1958)

(12) M. Stacey, Adv.Enzymology, 15, 301 (1954)

(13) B.C. Bera, A.B. Foster, M. Stacey, J.C.S., p. 3788 (1955)

(14) J.F. Douglas, C.G. King, J.Biol.Chem., 202, 865 (1953)

(15) W.F. Goebel, F.H. Babers, J.Biol.Chem., 100, 573 (1933); Chem.Abs., 27, 3450 (1933)

(16) W. Hach, D.G. Benjamin, J.A.C.S., 76, 917 (1954)

(17) S.K. Chanda, E.L. Hirst, E.G.V. Percival, J.C.S., p.1240 (1951)

(18) G.A. Adams, A.E. Castagne, Canad.J.Chem., 29, 109 (1951); Chem.Abs., 45, 8055 (1951)

(19) E.V. White, J.A.C.S., 68, 272 (1946)

(20) L. Hough, J.K.N. Jones, W.H. Wadman, J.C.S., p.796 (1952)

(21) G.O. Aspinall, Adv.Carbohydrate Chem., 9, 131 (1954)

(22) D.M.W. Anderson, N.J. King, private communication

(23) M.L. Wolfrom, R. Montgomery J.V. Karabinos, P. Rathgeb, J.A.C.S., 72, 5796 (1950)

(24) K. Meyer, M.M. Rapport, Science, 113, 596 (1951); Chem.Abs., 45, 8623 (1951)

(25) P. Hoffman, A. Linker, K. Meyer, Science, 124, 1252 (1956)

(26) K. Meyer, J.W. Palmer, J.Biol.Chem., 107, 629 (1934); Chem.Abs., 29, 2188 (1935)

(27) B. Weissmann, K. Meyer, J.A.C.S., 76, 1753 (1954); idem. J.Biol.Chem., 208, 417 (1954)

(28) L. Beauquesne, Compt.rend., 222, 1056 (1946); Chem.Abs., 40, 5279 (1946)

(29) S.P. James, F. Smith, J.C.S., pp.739, 746, 749 (1945)

(30) C.W. Woodmansee, G.L. Baker, Natural Plant Hydrocolloids, Adv. in Chemistry Series, American Chem.Soc., Washington, 1954, p.3

(31) F.A. Henglein, M. Hann, Makromol.Chem., 2, 289 (1948); Chem.Abs., 43, 5432 (1949)

(32) J. Ashby, J. Brooks, W.W. Reid, Chem.Ind., p.360 (1955)

(33) H.S. Isbell, H.L. Frush, J.Res.Nat.Bur.Stand., 32, 77 (1944); 33, 401 (1944)

(34) H.A. Spoehr, Arch.Biochem., 14, 153 (1947); Chem.Abs., 42, 932 (1948)

(35) F.G. Fischer, H. Dorfel, Z.physiol.Chem., 302, 186 (1955); Chem.Abs., 50, 7237 (1956)

(36) R.L. Whistler, K.W. Kirby, Z.physiol.Chem., 314, 46 (1959)

(37) A. Haug, Acta Chem.Scand., 13, 601 (1959)

(38) H.S. Isbell, H.L. Frush, J.Res.Nat.Bur.Stand., 31, 33 (1943)

(39) H.S. Isbell, H.L. Frush, J.Res.Nat.Bur.Stand., 32, 77 (1944)

(40) E. Schoeffel, K.P. Link, J.Biol.Chem., 100, 397 (1933); Chem.Abs., 27, 3451 (1933)

(41) F. Weinmann, Ber., 62B, 1637 (1929); Chem.Abs., 23, 5468 (1929)

(42) F. Ehrlich, K. Rehorst, Ber., 62B, 628 (1929); Chem.Abs., 23, 3666 (1929)

(43) E. Schoeffel, K.P. Link, J.Biol.Chem., 95, 213 (1932); Chem.Abs., 26, 2706 (1932)

(44) F.G. Fischer, H. Schmidt, Ber., 92, 2184 (1959)

(45) R.A. Edington, E. Percival, J.C.S., p.3554 (1955)

(46) E.L. Hirst, J.K.N. Jones, Modern Methods of Plant Analysis, Vol.II, Springer-Verlag, Berlin, 1955, p.275

(47) J.B. Pridham, Anal.Chem., 28, 1967 (1956)

(48) E.E.B. Smith, G.T. Mills, H.B. Bernheimer, R. Austrian, Biochim.Biophys.Acta, 29, 640 (1958)

(49) L.M. White, G.E. Secor, Anal.Chem., 31, 1273 (1959)

(50) H. Henecka, Houben-Weyl, Methoden der Organische Chemie; Verlag; Stuttgart, 1952, Vol.8, Part III, p.484

(51) B.R. Brown, Quart.Rev., 5, 131 (1951)

(52) E.D. Hughes, C.K. Ingold, J.C.S. p.244 (1935)

(53) H. Schenkel, M. Schenkel-Rudin, Helv., 31, 514 (1948); Chem.Abs., 42, 4525 (1948)

(54) E.J. Corey, J.A.C.S., 74, 5897 (1952)

(55) R.T. Arnold, O.C. Elmer, R.M. Dodson, J.A.C.S., 72, 4359 (1950)

(56) H. Schenkel, M. Schenkel-Rudin, Helv., 31, 924 (1948); Chem.Abs., 42, 5906 (1948)

(57) H.S. Wasserman, Steric Effects in Organic Chemistry, Editor: Newman; Wiley, New York, 1956, p.350

(58) R. Steinberger, F.H. Westheimer, J.A.C.S., 73, 429 (1951)

(59) P. Pratesi, L. Arpesella, A. LaManná, J.A.C.S., 75, 5476 (1953)

(60) C.C. Price, Mechanisms of Reactions at Carbon-carbon Double Bonds, Interscience Publishers, New York, 1946, p.55.

(61) F. Mann, B. Tollens, Annalen, 290, 155 (1896); Brit.Chem.Abs., 70, 417 (1896)

K.U. Lefèvre, B. Tollens, Ber., 40, 4513 (1907); Brit.Chem.Abs., 94, 74 (1908)

(62) A. Meller, Austral.J.Chem., 7, 157 (1954)

(63) B. Vollmert, Makromol.Chem., 3, 140 (1949); Chem.Abs., 44, 1855 (1950)

(64) W.E. Baier, Calif.Citrograph., 30, 378 (1945); Chem.Abs., 40, 410 (1946)

(65) E. Stutz, H. Deuel, Helv., 41, 1722 (1958)

(66) A.S. Perlin, Canad.J.Chem., 30, 278 (1952)

(67) G. Zweifel, H. Deuel, Helv., 39, 662 (1956)

(68) F.A.H. Rice, Abstracts 127th meeting Amer.Chem.Soc., Cincinnati, Ohio, 11E (1955) [in J.A.C.S., 78, 428 (1956)]

(69) H.S. Isbell, J.Res.Nat.Bur.Stand., 33, 45 (1944)

(70) G.L. Huber, H. Deuel, Helv., 34, 853 (1951);

G.L. Huber, Diss.E.T.H.Zurich, 1951

(71) S. Machida, Bull.Faculty Textile Fibers, Kyoto Univ., Japan, 1, 59 (1955)

(72) G. Zweifel, H. Deuel, Helv., 39, 662 (1956)

(73) F. Weber, Diss.E.T.H.Zurich, 1944, p.72 (Reference in 140)

(74) D.R. Nanji, F.J. Paton, A.R. Ling, J.Soc.Chem.Ind., (Trans.), 44, 253 (1925)

(75) F. Ehrlich, F. Schubert, Ber., 62B, 1974 (1929); Chem.Abs., 24, 65 (1930)

(76) A.D. Dickson, H. Otterson, K.P. Link, J.A.C.S., 52, 775 (1930)

(77) A.G. Norman, J.T. Martin, Biochem.J., 24, 649 (1930); Chem.Abs., 25, 1848 (1931)

(78) J.R. Bowman, R.B. McKinnis, J.A.C.S., 52, 1209 (1930)

(79) B. Burkhart, L. Baur, K.P. Link, J.Biol.Chem., 104, 171 (1934)

(80) R.L. Whistler, A.R. Martin, M. Harris, J.Res.Nat.Bur.Stand., 24, 13 (1940)

(81) W.V. Bartholomew, A.G. Norman, Chem.Abs., 36, 4442 (1942)

(82) E.C. Yackel, W.O. Kenyon, J.A.C.S., 64, 121 (1942)

(83) E.W. Taylor, W.F. Fowler, P.A. McGee, W.O. Kenyon, J.A.C.S., 69, 342, 355 (1947)

(84) M.V. Tracey, Biochem.J., 43, 185 (1948)

(85) B.L. Browning, TAPPI, 32, 119 (1949); Chem.Abs., 43, 3614 (1949)

(86) E. Letzig, Z.Lebensm.-Untersuch., 91, 325 (1950); Chem.Abs., 45, 1259 (1951)

(87) A.G. Ogston, J.E. Stanier, Biochem.J., 49, 591 (1951)

(88) A. Johansson, B. Lindberg, O. Theander, Svensk Papperstidning, 57, 41 (1954)

(89) H.W. Buston, Analyst, 57, 220 (1932)

(90) G. Lunde, E. Heen, E. Öy, Kolloid Z., 83, 196 (1938); Chem.Abs., 32, 6926 (1938)

(91) G.G. Maher, Anal.Chem., 21, 1142 (1949)

(92) R.M. McCready, H.A. Swenson, W.D. Maclay, Anal.Chem., 18, 290 (1946)

(93) S.A. Barker, A.B. Foster, I.R. Siddiqui, M. Stacey, Talanta, 1, 216 (1958)

(94) D.M.W. Anderson, Talanta, 2, 73 (1959)

(95) K.P. Link, C. Niemann, J.A.C.S., 52, 2474 (1930)

(96) E.A. Guggenheim, Phil.Mag., 2, 538 (1926)

(97) E.J. Conway, Microdiffusion Analysis and Volumetric Error; Crosby-Lockwood, London, 1947

(98) H. Thierfelder, Z.physiol.Chem., 11, 388 (1887); Brit.Chem.Abs., 52, 717 (1887)

(99) T. Reichstein, R. Oppenauer, Helv., 16, 988 (1933)

(100) C.M. Conrad, J.A.C.S., 53, 1999, 2282 (1931)

(101) D.M.W. Anderson, Talanta, 1, 283 (1958)

(102) P.P. Regna, B.P. Caldwell, J.A.C.S., 66, 246 (1944)

(103) F.A. Long, M.A. Paul, Chem.Revs., 57, 1, 935 (1957)

(104) J.N. Brönsted, Z.physik.Chem., 102, 169 (1922); Chem.Abs., 16, 4113 (1922)

(105) L. Zucker, L.P. Hammett, J.A.C.S., 61, 2791 (1939)

(106) C.K. Ingold, Structure and Mechanism in Organic Chemistry, Bell and Sons, Ltd., London, 1953, Chap.XIV

(107) L.P. Hammett, Physical Organic Chemistry, McGraw-Hill Inc., New York, 1940, Chap.IX

(108) A.I. Gel'bstein, G.G. Shcheglova, M.I. Temkin, Zhur.Neorg.Khim., 1, No.2, 282 (1956)

(109) W.M. Schubert, P.C. Myhre, J.A.C.S., 80, 1755 (1958)

(110) F.A. Long, J.G. Pritchard, F.E. Stafford, J.A.C.S., 79, 2362 (1957)

(111) J.G. Pritchard, F.A. Long, J.A.C.S., 80, 4162 (1958)

(112) H. Gilman, M.B. Lousinian, Rec.Trav.chim., 52, 156 (1933); Chem.Abs., 27, 2952 (1933)

(113) J.D. Cawley, D.R. Nelan, J.A.C.S., 77, 4130 (1955)

(114) R.M. Keefer, L.J. Andrews, R.E. Kepner, J.A.C.S., 71, 2381 (1949)

(115) H.A. Krebs, Biochem.J., 36, 303 (1942)

(116) F. Lynen, H. Scherer, Annalen, 560, 164 (1948)

(117) S. Ochoa, J.Biol.Chem., 174, 115 (1948)

(118) J.F. Speck, J.Biol.Chem., 178, 315 (1949)

(119) L.O. Krampitz, C.H. Werkman, Biochem.J., 35, 595 (1941)

(120) R. Steinberger, F.H. Westheimer, J.A.C.S., 71, 4158 (1949)

(121) J.E. Prue, J.C.S., p.2331 (1952)

(122) A. Albert, Biochem.Soc.Symposium No.15, "Metals and Enzyme Activity", Editor: E.M. Crook; Cambridge Univ. Press, 1958, p.48.

(123) A. Kornberg, S. Ochoa, A.H. Mehler, J.Biol.Chem., 174, 159 (1948)

(124) A.E. Martell, M. Calvin, Chemistry of the Metal Chelate Compounds, Prentice-Hall Inc., New York, 1952.

(125) A. Schellenberger, Z.physiol.Chem., 309, 16 (1957); Chem.Abs., 52, 11748 (1958)

(126) K.J. Pedersen, Acta Chem.Scand., 3, 676 (1949)

(127) K.J. Pedersen, Acta Chem.Scand., 6, 285 (1952)

(128) E. Gelles, J.P. Clayton, Trans.Faraday Soc., 52, 353 (1956)

(129) E. Gelles, J.Inorg.Nuclear Chem., 8, 625 (1958)

(130) R.J.P. Williams, Nature, 171, 304 (1953)

(131) H. Lück, Z.Lebensm.-Untersuch., 106, 1 (1957); Chem.Abs., 51, 16992 (1957)

(132) J.F. Scaife, Canad.J.Biochem.Physiol., 37, 1049 (1959)

(133) K.J. Pedersen, Acta Chem.Scand., 9, 1640 (1955)

(134) V. Franzen, L. Fikentscher, Annalen, 613, 1 (1958); Chem.Abs., 52, 18314 (1958)

(135) R.F. Nickerson, Ind.Eng.Chem., Anal.Edn., 13, 423 (1941)

(136) W. Voss, J. Pfirschke, Ber., 70B, 631 (1937); Chem.Abs., 31, 4620 (1937)

(137) K. Freudenberg, H. Gudjons, G. Dumpert, Ber., 74B, 245 (1941) Chem.Abs., 35, 3969 (1941)

(138) O. Ruff, Ber., 31, 1573 (1898); Brit.Chem.Abs., 74, 516 (1898)

(139) H.G. Fletcher, H.W. Diehl, C.S. Hudson, J.A.C.S., 72, 4546 (1950)

(140) G. Zweifel, Diss.E.T.H.Zurich, 1956 [see also ref.(72)]

(141) A.F. Trotman-Dickenson, Gas Kinetics, Butterworths Scientific Publications, 1955, p.33

(142) R.W. Taft, E.L. Purlee, P. Riesz, C.A. DeFazio, J.A.C.S., 77, 1584 (1955)

(143) Personal communication, Dr. R. Scott, Biophysics Dept., University of Edinburgh

(144) J. Bjerrum, G. Schwarzenbach, L.G. Sillén, Stability Constants; Part I, Organic Ligands. Chemical Society Special Publication No.6., The Chemical Society, London, 1957.

(145) C.G. Swain, J.A.C.S., 72, 4578 (1950)

(146) E.J. McDonald, J.Org.Chem., 25, 111 (1960)

(147) D.T. Sawyer, R.S. George, T.B. Bagger, J.A.C.S., 81, 5893 (1959)

(148) R.E. Reeves, Adv.Carbohydrate Chem., 6, 107 (1951)

(149) H. Franken, Biochem.Z., 257, 245 (1933); Chem.Abs., 27, 1881 (1933)

(150) G.A. Guanzon, W.M. Sandstrom, Analyst, 63, 130 (1938)

(151) W.G. Campbell, E.L. Hirst, G.T. Young, Nature, 142, 912 (1938)

(152) A.G. Norman, Nature, 143, 284 (1939)

(153) K.P. Link (1931). Quoted in Anderson (1931) - ref.(154)

(154) E. Anderson, J.Biol.Chem., 91, 559 (1931)

(155) H.A. Spoehr, H.W. Milner, J.Biol.Chem., 111, 679 (1935)

(156) H. Colin, S. Lemoyne, Bull.Soc.Chim.biol., 20, 343 (1938)

(157) H. Colin, S. Lemoyne, Bull.Ass.Chim.Sver., 55, 433 (1938)

(158) W.H. Fuller, W.V. Bartholomew, A.G. Norman, Soil Sci., 64, 143 (1947); Chem.Abs., 41, 7607 (1947)

(159) I.E. Puddington, Canad.J.Res., B26, 415 (1948); Chem.Abs., 42, 6758 (1948)

(160) H.S. Isbell, J.Res.Nat.Bur.Stand., 32, 45 (1944)

(161) M.L. Wolfrom, R.D. Schuetz, L.F. Cavalieri, J.A.C.S., 70, 514 (1948); 71, 3518 (1949)

(162) B. Singh, G.R. Dean, S.M. Cantor, J.A.C.S., 70, 517 (1948)

(163) E. Pacsu, L.A. Heller, J.A.C.S., 70, 523 (1948)

(164) J. Schurz, Svensk Papperstidn., 59, 98 (1956)

(165) F. Bandow, Biochem.Z., 294, 124 (1937); Chem.Abs., 32, 1181 (1938)

(166) F. Petuely, Monatsh.Chem., 84, 298 (1953); Chem.Abs., 48, 4447 (1954)

(167) C.D. Hurd, L.L. Isenhour, J.A.C.S., 54, 317 (1932)

(168) W.N. Haworth, W.G.M. Jones, J.C.S., p.667 (1944)

(169) O. Hassel, B. Ottar, Acta Chem.Scand., 1, 929 (1947)

(170) W.N. Haworth, J. Jackson, F. Smith, J.C.S., p.620 (1940)

(171) C. Tanaka, Mem.Coll.Sci., Kyoto Imp.Univ., 13A, 239, 265 (1930); Chem.Abs., 24, 5025 (1930)

(172) M. Cifonelli, J.A. Cifonelli, R. Montgomery, F. Smith, J.A.C.S., 77, 121 (1955)

(173) M.L. Wolfrom, E.G. Wallace, E.A. Metcalf, J.A.C.S., 64, 265 (1942)

(174) R.M. Love, Biochem.J., 55, 126 (1953); 56, 639 (1954)

(175) W.N. Haworth, E.L. Hirst, V.S. Nicholson, J.C.S., p.1513 (1927)

(176) F.A.H. Rice, L. Fishbein, J.A.C.S., 78, 1005 (1956)

(177) F.A.H. Rice, L. Fishbein, J.A.C.S., 78, 3731 (1956)

(178) E. Votoček, S. Malachta, Coll.trav.chim.tchéceslov., 6, 241 (1934); Chem.Abs., 29, 469 (1935)

(179) F. Ehrlich, R. Guttmann, Ber., 67, 573 (1934); Chem.Abs., 28, 3717 (1934)

(180) E.G. Young, F.A.H. Rice, J.Biol.Chem., 164, 35 (1946)

(181) A.M. Gakhokidze, J.Gen.Chem.(U.S.S.R.), 11, 109 (1941); Chem.Abs., 35, 5464 (1941)

(182) D.M.W. Anderson, Analyst, 84, 50 (1959)

(183) G.F. Davidson, Shirley Inst.Mems., Vol.XXI, IV-IX, Part II, (1947)

(184) H.L. Yale, Chem.Revs., 33, 209 (1943)

(185) M.A.G. Kaye, P.W. Kent, J.C.S., p.79 (1953)

(186) R. Hilf, F.F. Castano, Anal.Chem., 30, 1538 (1958)

(187) O.G. Lien, Anal.Chem., 31, 1363 (1959)

(188) B. Tollens, Ber., 41, 1788 (1908); Brit.Chem.Abs., 94, 639 (1908)

(189) C. Neuberg, M. Kobel, Biochem.Z., 243, 435 (1931)

(190) A. Grauer, C. Neuberg, Anal.Chim.Acta, 8, 422 (1953)

(191) A.M. Venet, J. Pouradier, J.M. Landucci, Bull.Soc.Chim.France, p.1325 (1957)

(192) C.A. Browne, F.W. Zerban, Sugar Analysis, 3rd Edn., John Wiley and Sons, Inc., New York, 1955, pp.653-738

(193) J.R. Helbert, K.D. Brown, Anal.Chem., 31, 1700 (1959)

(194) J.S. Hepburn, M. Lazarchick, Am.J.Pharm., 102, 560 (1930); Chem.Abs., 25, 264 (1931)

(195) Z. Dische, J.Biol.Chem., 167, 189 (1947); 183, 489 (1950)

(196) L. Federico, M. Ciucani, Chim e industria, Ital., 36, 598 (1954)

(197) S.M. Stark, Anal.Chem., 22, 1158 (1950)

(198) E.A. McComb, R.M. McCready, Anal.Chem., 24, 1630, 1986 (1952)

(199) J.M. Bowness, Biochem.J., 67, 295 (1957)

(200) P. Dubach, D.L. Lynch, Soil Science, 87, 273 (1959)

(201) K. Heyns, W. Baltes, Ber., 91, 622 (1958)

(202) H.G. Bott, E.L. Hirst, J.C.S., p.2621 (1932)

(203) J.M. Bowness, Biochem.J., 67, 295 (1957); 70, 107 (1958)

(204) A.H. Guerrero, R.T. Williams, Nature, 161, 930 (1948)

(205) T. Momose, Y. Ueda, M. Iwasaki, Pharm.Bull.Japan, 4, 49 (1956)

(206) H. Neukom, P. Hui, Chimia, 13, 330 (1959)

(207) D.M.W. Anderson, J.L. Duncan, in press

(208) K. Aso, Tohuku J.Agr.Res., 3, 359 (1953)

(209) E. Fischer, Ber., 27, 1524 (1894); Brit.Chem.Abs., 66, 395 (1894)

(210) E. Votoček, S. Malachta, Coll.trav.chim.tchécoslov., 8, 66 (1936); Chem.Abs., 30, 3410 (1936)

(211) G. Chavanne, Ann.Chim.Phys., 8, 507 (1904); Brit.Chem.Abs., 88, 77 (1905)

(212) P.A. Yoder, B. Tollens, Ber., 34, 3446 (1901); Brit.Chem.Abs., 82, 49 (1902)

(213) H.B. Hill, Amer.Chem.J., 25, 440 (1901); Brit.Chem.Abs., 80, 555 (1901)

(214) A.P. Dunlop, F.N. Peters, The Furans, Reinhold Publishing Corp., New York, 1953, pp.389-395.